Rapid Literature Review

Influenza A and Dairy Cattle

April 2024

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RAPID LITERATURE REVIEW - INFLUENZA A AND DAIRY CATTLE

INTRODUCTION

There is currently limited information available on the susceptibility of cattle to influenza A viruses (IAV). The susceptibility of cattle to avian IAVs in particular is not well understood and there are no published studies on cattle and the currently circulating H5N1 Eurasian lineage clade 2.3.4.4.b panzootic strain. However, throughout recent history, it has been noted that some cattle populations have shown signs of illness during outbreaks of influenza A in people (Sreenivasan et al., 2019) and a few research studies are available that investigate the susceptibility of cattle to human strains of IAVs in particular. Additionally, some experimental studies are available that investigate the susceptibility of cattle or related ruminants to other influenza A strains.

METHOD

This scientific literature review includes information related to influenza A infection in cattle. The literature search by the Canadian Agriculture Library used Scopus and CAB, and included the following search strategy: (dairy OR cattle OR cow OR ruminant) AND "influenza A" OR hpai OR "highly pathogenic avian influenza " OR "bird flu"(OVID search was limited to last 3 years). An additional search was also conducted using the Canadian Agriculture Library website using a similar combination of key words, including cattle, cow, or ruminant and influenza A, highly pathogenic avian influenza, and bird flu. Finally, additional relevant articles were also found through the 2019 review article by Sreenivasan et al.

NATURAL INFECTION/OUTBREAK STUDIES

The most recent study available was conducted in **2013** by **El-Sayed et al.** who investigated the seroprevalence of avian influenza in animals and humans in Egypt. Sera was collected from humans and various animals including ruminants such as cattle, buffalo, sheep and goats in Cairo and the surrounding governorates to examine the presence of anti-H5N1 antibodies. Sera from 50 cattle were included with positive controls obtained from experimentally vaccinated animals. The hemagglutination-inhibition (HI) test using inactivated antigen from local H5N1 Egyptian field isolates (Chicken/Egypt/9402-NAMRU3-CLEVB213/2007) and a protein G modified H5N1 enzyme-linked immunosorbent assay (ELISA) that detects antibodies against the H5 of the IAV in bird sera were performed, both resulting in no detection of anti-H5N1 antibodies in any of the ruminants (n=200).

Some of the most extensive studies on influenza A in cattle have been conducted in the United Kingdom (UK) with investigations into two human IAV strains, H1N1 (A/England/333/80) and H3N2 virus (A/England/427/88). **Brown et al. (1998)** detected antibodies to IAV in cattle in association with bovine respiratory disease (BRD) and reduced milk yield in two unrelated outbreaks of BRD on two farms in Great Britain in February and March 1998. Farm A had 100 per cent morbidity (BRD) and no mortality. BRD on Farm B was more sporadic and mainly affected milking cows. Clinical signs on the farms included increased respiratory rate and lung sounds, anorexia and, in milking cows, severely reduced milk yields. Many affected animals were pyrexic and a proportion of them had ocular/nasal discharge, loose faeces and a cough. Both farms demonstrated rising antibody levels in most animal sera to human H1N1 and H3N2 through HI testing. Type specific antibodies to IAV in cattle on Farm A were confirmed though the gel diffusion precipitation test. Virus neutralisation (VN) test results also showed clear positive VN titres on both farms. Rising antibody levels to bovine respiratory syncytial virus were found in all cattle on Farm A.

Antibody levels on Farm B were static. Further investigation of antibodies to human IAV in cattle was conducted using 500 randomly selected sera that had been submitted to the Central Veterinary Laboratory in winter 1997-98 for investigation of respiratory disease and comprised of 110 incidents. HI antibodies to human H1N1 (202 seropositives) and/or human H3N2 (239 seropositives) were detected in 244 (49%) sera derived from 71 incidents (65%).

In a small year-long survey by the Veterinary Laboratories Agency Langford (UK) beginning in summer of 1997 by **Gunning et al. (1999)** Holstein Friesian dairy herds with a recent history of sporadic milk drop were examined clinically with collection of blood samples for routine haematological and biochemical investigation. Convalescent blood samples were collected at least two weeks later. Paired sera from all 45 cows (in five herds ranging from 120 to 200 cows) were tested for various common pathogens using various tests for pathogen detection. The failure to detect significant numbers of seroconversions to any of the pathogens tested suggested they were not major contributors to the observed sporadic milk drop syndrome. Following recent identification of antibodies to IAVs in cattle in Great Britain (see Brown et al., 1998), paired sera from 40 of the cows were further tested by HI using specific human IAV strains. There were significant rises in antibody titre to H1N1 and H3N2 in 24 (60%) and 26 (65%) cows, respectively, and this response was detected in at least two cows in each of the five herds examined. Only two (5%) of the cows tested were seronegative to both H1N1 and H3N2 viruses.

Graham et al. (2002) conducted a retrospective analysis for evidence of IAV infection in cattle in Northern Ireland. Isolation of IAV from nasal mucus or swab samples from 142 archived samples from 46 cases of respiratory disease and/or milk drop syndrome was unsuccessful. However, HI testing found antibodies to human IAVs present in collected sera. Specifically, 84 pairs of acute and convalescent serum samples were collected in 1998 and 1999 from 17 outbreaks of respiratory disease, milk drop syndrome or diarrhea in cattle. Antibodies to H1N1 and H3N2 were present in the convalescent sera of 56.5% and 58.8% cattle tested, respectively, with 56% of the animals seroconverting to one or both viruses. The titres to H3N2 were often several times higher than those to H1N1. Additional testing of a selected subset of 21 sera against six further strains of H1N1 and H3N2 from human (H1N1 strains A/Johannesburg/82/96 and A/Beijing/262/95, and H3N2 strains A/Sydney/5/97 and A/Nanchang/933/95) and porcine (H1N1 strain A/sw/England/195852/92 and H3N2 strain A/sw/Stormont/387/86) influenza viruses currently circulating in Northern Ireland pig herds also revealed that the highest reactivity was to human H3N2 strains. The titres to human H1N1 strains and to both porcine subtypes were low or absent. The markedly greater reactivity with the H3N2 than with the H1N1 subtypes differs from the findings of Brown et al. (1998), who observed a broad cross-reactivity with human H1N1 and H3N2 strains.

In **Crawshaw et al. (2008)** two Holstein Friesian dairy herds in UK were investigated where cows had been affected sporadically with an acute fall in milk production with return to normal production in 10–14 days. The study design was a case control study with two matched controls per case. The cases were matched for lactation (but not for lactation stage) as there was some evidence from previous work that cows in their first and second lactations were more often affected with an acute fall in milk production than those in later lactations. For each case two control cows in the same lactation and from the same management group were selected. All three were blood sampled and clinically examined as soon as possible, and second blood samples were taken a median of 21 days after the first samples. A HI test was carried out on the sera as a single batch using the two human IAVs (H1N1 and H3N2). ELISA tests for common bovine pathogens were also carried out on the stored sera as a single batch. The paired blood samples demonstrated that rising antibody titres to H1N1 and H3N2 were associated with pyrexia, increased

respiratory noise, and an acute fall in milk production in cattle, which amounted to a mean loss of 159.9 L of milk for affected cases compared to the controls. This equates to about 2.0% of the lactation yield of a cow in this herd. Rising titres to common bovine pathogens were not associated with an acute fall in milk production. In most cows these were rises in antibody titre rather than seroconversions suggesting pre-existing immunity. Cases with rises in antibody to IAV had significantly higher respiratory scores and rectal temperatures than their controls.

A study has also been conducted in cattle in the United States (Minnesota) on an H1N1 human influenza A strain. A retrospective study by **Jones-Lang et al. (1998)**[1] investigated the prevalence of IAV (H1N1) antibodies in 2,345 bovine sera through an H1 subtype-specific indirect ELISA. The findings showed that 27% of the samples tested were positive, 31% were low-positive and 42% were negative. The prevalence of antibody appeared to peak during the months of September to November and then again from February to March. A subset of the above samples was examined by HI test to confirm the ELISA results. A 92% correlation was found between the ELISA and HI assay. Western blot analysis on a subset of ELISA positive sera (n=50) confirmed the presence of antibodies to the nucleoprotein and H1 hemagglutinin protein of IAV.

A serological study on susceptibility of animals in Nepal and India to influenza was conducted by **Graves et al. (1974)**. Naturally-occurring antibody to H3N2 (X-31 strain of A/Hong Kong/1/68) human influenza antigens was found in cattle (6 positive/9 tested) and water buffaloes (2/2) in 2 locations in Kathmandu, and goats (5/12) and cattle (10/19) in 2 villages in West Bengal, India, as shown by positive radial diffusion (RD) tests (that provide a sensitive method for measuring the relative concentrations of antibody). The specificity of water buffalo sera in this test remains to be studied. A seroconversion in a 6-month-old calf in India was also observed.

Lastly, the only study to isolate an IAV from a naturally infected calf was conducted by **Fatkhuddinova et al. (1973)**[2] that involved serologic and virologic surveys of cattle in Tajikistan between 1970 and 1972. An influenza virus was isolated from a sick calf and identified as the Hong Kong variant A/calf/Dushanbe/55/72 (H3N2). The circulation of this strain was proven by detection of antibodies in the blood sera of domestic cattle by the complement fixation test and the HI test. A total of 588 blood serum specimens of cattle were examined and HI antibodies against H3N2 were found in 12.07% of cattle sera.

EXPERIMENTAL STUDIES

Cattle have also been found to be susceptible to infection with IAVs experimentally, including to a virus strain closely related to the currently circulating highly pathogenic avian influenza (HPAI) H5N1 panzootic strain. In a study at the Friedrich-Loeffler-Institut in Germany in 2007, four three-month old Holstein-Friesian calves were inoculated intranasally with 5ml of 50% egg infectious dose (EID)/ml HPAIV (H5N1) strain A/cat/Germany/R606/2006 (Asian lineage) and monitored for seven days (**Kalthoff et al., 2008**). Two other calves were kept in the same room to investigate transmission. At 1 day post-inoculation (dpi), virus was detected by PCR in nasal swabs from all four inoculated calves, with infectious virus in three of the four calves. At 2 dpi, virus was detected by PCR in two of the four inoculated calves, with infectious virus detected in one of the four calves. No viral shedding was detected after 3 dpi. At 14 dpi, antibodies

[1] Only abstract available at this time
[2] Only abstract available in English

to the virus were detected in two of the four inoculated calves by ELISA and at 28 dpi, antibodies were detected in all four calves by HI. None of the calves showed any clinical signs of illness. In the contact calves, virus was not detected in nasal swabs at any point in the study, however, at 21 dpi, antibodies to the virus were detected in one of the two contact calves by VN. This calf did not show any clinical signs of illness.

Other experimental studies have investigated the susceptibility of cattle to influenza A strains from humans, a calf, swine, and horses. In a study in Hong Kong in 1977, three Holstein calves were inoculated intranasally with three different human influenza A strains, one strain into each calf (**Campbell et al., 1977**). Additionally, two Holstein calves were inoculated intranasally with a strain isolated from a calf (A/calf/Duschambe/55/71, thought to have been a variant of a human strain). None of the calves inoculated with the human strains developed any clinical signs of illness. However, at 3-4 weeks after inoculation, two of the calves developed low level antibodies. In contrast, the calves inoculated with the strain isolated from the calf developed nasal discharge and rhinitis. At 3-4 weeks after inoculation high level antibodies were also detected.

A study in the US in 2010 examined the susceptibility of cattle to equine influenza H3N2 (**Lin et al., 2010**). First, virus was inoculated into bovine respiratory epithelium *in vitro* where it was found to replicate. Six beef calves were then exposed to 10^6 EID50 aerosolized influenza A/equine/Kentucky/91 (H3N8) and nasopharyngeal swabs were collected daily for eight days. None of the calves developed any clinical signs of illness. Additionally, no infectious virus was detected in the swabs at any point. Sera was also collected from the calves at 0 dpi, 9 dpi, and 20 dpi, and tested for antibodies to the virus by HI, but no antibodies were detected.

Nakamura and Easterday (1967) investigated non-specific inhibitor substances thought to be present in cattle that may play a role in their susceptibility to influenza infections. Thirty Holstein cattle, including 10 calves, 10 yearlings, and 10 cows, were injected intravenously with an influenza A strain (A/PR8/34) and serum was collected at various times. Serum samples were heat treated to try and remove non-specific inhibitor substances. A primary-type antibody response was detected in the majority of animals, but high level antibody titres were not achieved through HI testing.

In another experimental study by **Lopez and Woods (1987)**[3] calves were inoculated intranasally with live swine influenza virus (SIV) A/sw/IL/75 (H1N1). SIV was isolated for 7 days and respiratory tract disease was observed. Antibody was detected in serum of inoculated calves from 9 dpi, and VN antibody was demonstrated on 14 and 21 dpi. The primary response was low. At necropsy pneumonic lesions and histopathologic changes were observed in airways and lungs. Fluorescent staining revealed viral activity in epithelial cells of airways. The study also found that virus transferred to healthy calves housed with inoculated calves.

A Canadian study investigated influenza A in goats as a surrogate for cattle (**Mitchell et al., 1956**). Goats were injected with 2ml of 10^{-7} influenza A (PR 8 strain) directly into the mammary gland. Viral titers increased and were detectable for approximately 10 days after inoculation after which neutralizing antibodies developed in both the milk and blood. The part of the mammary gland that was injected was

[3] Only abstract available at this time

then surgically removed and it was noted that although the neutralizing antibody titre decreased, a high level of antibodies remained for several months.

The previously mentioned serological study on susceptibility of animals in Nepal and India to influenza conducted by **Graves et al. (1974)** also included experimental inoculation of seronegative yak with human H3N2 IAVs (A/Hong Kong/1/68 and A/England/42/72) and equine IAV (A/equine/Prague/1/56). Serial administration of the viruses were carried out intranasally and intratracheally and HI and RD tests were conducted. The primary antibody response to infection with A/Hong Kong/1/68 virus occurred within 9 dpi while mild respiratory symptoms, coughing and malaise were observed on the sixth day. The titre of A/England/42/72 increased in 6 days and reached the level seen with A/Hong Kong/1/68 antibody at 23 dpi. No respiratory symptoms were observed after the A/England/42/72 challenge. For the equine IAV, malaise and anorexia were observed on the 5th day, and specific antibody was detected on the 7th day.

SUMMARY

In summary, cattle have been found to be susceptible to some strains of influenza A virus and in some cases infection may cause disease. Research suggests that cattle are susceptible to the human IAVs H1N1 and H3N2 and that infection may be more widespread than currently thought. These virus strains also likely play an important role in causing respiratory disease and milk drop syndrome in cattle. Experimentally, cattle have also been found to be susceptible to a HPAI H5N1 strain and infected calves may be able to spread this virus in the absence of any clinical signs. Cattle may also be susceptible to other strains of influenza A viruses, including some swine and equine strains, however, this appears to greatly depend on the particular strain. Overall, there has been limited research on IAVs in cattle, particularly on avian influenza virus strains. Further research is needed in order to better understand the susceptibility of cattle to different IAVs, the pathogenesis of disease in infected animals, and their role in the transmission of these viruses.

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