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RESEARCH ARTICLE

Hepatitis associated with hepatitis B virus in broilers

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Abstract

Infection by hepatitis B virus (HBV) results in acute and chronic liver damages in humans. Liver products of broilers as a primary food consumed in our daily life have a close connection with public health. The prevalence of the virus in livers and serum of broilers is of great significance, owning to the potential transmission between chickens and humans. Liver tissues and serum samples were tested to investigate the prevalence of hepatitis B virus infection in slaughtered broilers, for expression of HBV antigens and antibodies. The distribution and positive rate of hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg) in liver samples were examined using immunohistochemistry. HBsAg was mainly located in the cytoplasm of hepatocytes with a positivity of 81.61% whereas HBeAg and HBcAg were primarily located in the nucleus of hepatocytes with a positivity of 40.13 and 49.10%, respectively. Enzyme-linked immunosorbent assay (ELISA) analysis of serum for HBV serological markers demonstrated a high prevalence of hepatiits B surface antibody (HBsAb, 54.91%) and hepatitis B core antibody (HBcAb, 27.68%), whereas HBeAb, HBsAg and HBeAg were rarely detectable. Classic hepatitis pathological changes, including swollen hepatocytes, focal parenchymal necrosis, lymphocytic infiltration and hyperplasia of fibrous connective tissues were observed using histopathological analysis. Some of the liver samples were found positive for HBV DNA using nested PCR. Sequence comparison confirmed that all sequences shared 97.5–99.3% identity with human HBV strains. These results demonstrated the existence of HBV in livers and serums of broilers. Animals or animal products contaminated with HBV could raise an important public health concern over food safety and zoonotic risk.

Keywords: broilers, hepatitis B, virus detection, prevalence investigation

1. Introduction

Hepatitis B virus (HBV), the causative pathogen of acute and chronic hepatitis B, is responsible for important public health disease worldwide (Seeger and Mason 2000; Ganem and Prince 2004). It is estimated that over 350 million people have been chronically infected, especially in Asian and African countries (Rossi *et al.* 2012), and those individuals are at high risk of developing liver cirrhosis and

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hepatocellular carcinoma (HCC) (Feitelson 1999; Tarocchi 2014). The infectious condition of patients with HBV is generally evaluated by detecting serological markers including hepatitis B surface antigen (HBsAg), hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), hepatiits B surface antibody (HBsAb), and hepatitis B core antibody (HBcAb). All the serological markeres are crucial for clinical diagnosis of HBV in humans (Raimondo *et al.* 2003). HBV surface proteins are important in viral immunity, and the detection of HBsAg in serum is fundamental diagnostic method used to evaluate HBV infection. Besides, loss of HBeAg and developing anti-HBe antibodies namely HBeAg seroconversion, are associated with lower viral replication level. In addition, high serum HBsAg levels can predict fibrosis among HBeAg-positive patients in a certain degree (Seto *et al.* 2012).

Hepatitis B virion is reported to be an enveloped, hepatotropic virus that is 40 to 42 nm in diameter (Dane *et al.* 1970) with a partly double-stranded and relaxed circular DNA genome approximately 3.2 kb. HBV is a member of the Hepadnaviridae family. There are two recognized genera: the genus *Orthohepadnavirus*, which is responsible for the infection of mammals and the genus *Avihepadnavirus*, which contains species that infect birds. So far, *Orthohepadnaviruses* have been identified in woodchucks, ground squirrels and primates including woolly monkeys, orangutans, gorillas and gibbons (Lanford *et al.* 1998; Warren *et al.* 1999; Lanford *et al.* 2000; MacDonald *et al.* 2000). The first report of *Avihepadnavirus* naturally infection was confirmed in Peking ducks (Marion *et al*. 1984). Subsequently, accumulating research proved the existence of HBV-like viruses isolated from other avian species such as the grey heron, Ross' goose, snow goose and white stork (Sprengel *et al.* 1988; Chang *et al.* 1999; Pult *et al.* 2001; Prassolov *et al.* 2003; Guo *et al.* 2005). Most recently, Hepadnaviridae viruses have been discovered in bats and parrots (Piasecki *et al.* 2012; Drexler *et al.* 2013; He *et al.* 2013; Piasecki *et al.* 2013). Considerable evidence indicates the expansion of the potential animal host range of HBV-like viruses and the emerging risk of hepatitis B. Nevertheless, the pathogenesis of HBV and transmission between human and primates or other animals have not been confirmed or explicitly explained.

Because of the daily consumption of chicken and liver products, the latter are closely associated with public health. Furthermore, recent research has found the existence of viruses similar to HBV (HBV-like virions) in food animals such as pigs (Li *et al.* 2010), with virions observed using transmission electron microscopy (TEM) and HBV DNA amplified by PCR, which suggested that the possibility of food animal like swine infected with HBV. However, whether animal HBV could be transmitted through a foodborne pathway to humans still needs to be elucidated. In this study, we determined the serological prevalence of hepatitis B in slaughtered broilers to evaluate HBV infection. This work may provide a new perspective on animal HBV and public health.

2. Results

2.1. Morphologic assessment of liver damage

Gross appearance including enlargement of the liver, sporadic tiny necrotic foci on the liver surface and yellowish color were observed at autopsy (data not shown). Histopathological changes similar to human hepatitis were observed and shown in Table 1. The predominant pathological changes were swollen hepatocytes with extensive cytoplasmic vacuolization, fatty degeneration and focal parenchymal necrosis. Swelling liver samples with yellowish color always demonstrated edema and small to large sizes of lipid droplets in hepatocytes microscopically. Necrotic foci were consistent with degeneration and necrosis of hepatocytes under microscope. Inflammatory cells especially lymphocytes, were commonly observed between hepatocytes and around portal areas. Furthermore, biliary hyperplasia and proliferation of bile duct epithelium were found in some samples, which were somewhat turgid at gross observation. Other lesions, including edema, congestion and cholestasis, were also seen in sporadic cases.

Table 1 Histopathological changes observed in liver samples

Histopathological changes	Samples (*n*)	Rates (%)
Edema	233	52.24
Congestion	192	43.05
Degeneration or necrosis	403	90.36
Infiltration of lymphocytes	389	87.22
Bile ductal hyperplasia	212	47.53
Proliferation of bile duct epithelium	110	24.66
Fibrous connective tissue proliferation	341	76.46
Cholestasis	23	5.16

Collagen fibers were stained blue with Mallory's trichrome. Mallory's trichrome staining of liver samples showed obvious proliferation of fibrous connective tissues and abundant hyperplasia of reticular fibers, especially around portal areas and even between hepatocytes (Fig. 1-E and F), with an incidence of 76.46% (Table 1).

2.2. Immunohistochemical (IHC) analysis

Expression of HBsAg, HBcAg and HBeAg was estimated using immunohistochemical (IHC) staining (Table 2). As