THE EFFECT OF DUCK HEPATITIS B VIRUS ON PERSISTENT INFECTION ON LAYING PERFORMANCE IN BROWN TSAIYA DUCKS

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Summary

In order to understand the effect of duck hepatitis B virus (DHBV) on the economic performance of ducks, three groups (DHBV congenitally infected, experimentally infected and DHBV negative) Brown Tsaiya ducks (*Anas platyrhyncha*) were used for experimental animals. Artificial insemination and pedigree hatching were applied in the propagation of ducklings, and the efficiency of vertical transmission and experimental infection was analyzed through the detection of DHBV DNA in the sera of 8-week-old offspring. The observation of the records of the first year indicated that the persistent infection had no significant effects on the performance of ducks, except the egg number of survival ducks up to 40 week of age. Thus DHBV infection did not appear to give ill effects to the economic performance of ducks in first laying year. A higher infection rate (85.3%) was obtained in congenital transmission than that (75.5%) of experimental infection. Both modes of infection did not reach 100% infectious rate, although some ducks developed transient viraemia in a tracing of DHBV DNA for 24 weeks to 11 challenged ducklings.

(**Key Words**: Duck Hepatitis B Virus, Vertical Transmission, Experimental Infection, Laying Performance)

Introduction

The duck industry is one of the important agricultural enterprises in Taiwan. The total annual value of duck products is over US$ 300 million per year. This includes meat ducks, processed duck eggs, exported half-incubated eggs, frozen duck meat, and other by-products, mainly duck feathers (Tai, 1985). Duck virus hepatitis A type had seriously happened about twenty years ago in this country, however, it was controlled quite well by vaccination and antisera. Duck hepatitis B virus (DHBV) was isolated from commercial duck flocks in the United States early in the 1980s (Mason et al., 1980), and it was also detected from commercial duck farms in Taiwan (Chang et al., 1987). Although DHBV may induce chronic disease to ducks, there is few study about the effects of DHBV on the economic performance of ducks. Thus the current purpose of the present study is to find out that whether the DHBV infection will cause serious effects on the economic production in ducks. In addition, the duck has been recently used as an animal model in the research of human hepatitis B virus (HBV), because the duck hepatitis B virus has been classified as a member of hepadnaviridae as HBV (Mason et al., 1980). In Taiwan, the study of the genetic effects of ducks on their response to DHBV is therefore considered to be an ideal animal model for HBV due to the large population and well-developed system of artificial propagation in ducks. Thus the long term purpose of this project is to select duck lines for genetically resistant and susceptible to DHBV in order to supply laboratory animals for animal model researches.

Materials and Methods

Animals. Two hundred and eighty nine 30-week old Brown Tsaiya ducks (79♂ and 210♀) were obtained from a commercial duck farm. Four drakes and twelve ducks were confirmed as viraemia ducks by spot hybridization assay. Twelve DHBV-positive ducks were artificially inseminated with semen collected from 5 drakes (2 DHBV + and 3 DHBV −) to produce 136 ducklings. Meanwhile 21 DHBV - negative ducks

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were also artificially inseminated with semen collected from 6 drakes (2 DHBV + and 4 −) to produce 171 ducklings. All ducklings were underwent by pedigree hatching. Forth five ducklings derived from parents with DHBV-negative were injected with 200 μl of DHBV-positive serum (contained more than 6×10^6 virions per ml) within 24 hours after hatching. Sera of all ducks were collected at 8 weeks and 47 weeks of age for DHBV DNA assay. Eleven out of 45 challenged ducklings were traced DHBV DNA in serum each week up to 24 weeks of age.

Management. All ducklings were separated as expected DHBV-positive and negative at 24 hours after hatched and raised in different battery brooders up to 6 weeks of age. All ducks were raised in floor pens during 7-16 weeks of age. After 16 weeks of age, ducks were kept in individual cages. DHBV-negative ducks confirmed by spot hybridization assay at 8 weeks of age were kept in different rows of cages in order to get rid of contamination from DHBV-positive ducks. Feeds and water were supplied *ad lib*. The crude protein (CP) and metabolizable energy (ME) of feeds were as follows: before 6 weeks of age, CP 19% and ME 2,900 Kcal/Kg: 7-16 weeks of age, CP 14% and ME 2,800 Kcal/Kg; after 16 weeks of age, CP 20% and ME 2,800 Kcal/Kg.

Performances test. The body weights at growing stage were obtained at 2, 4, 6, 8, 10 and 12 weeks of age respectively. The laying performances were recorded, including the age of sexual maturity (age at first egg), egg weight at sexual maturity and 45-week-old, egg number of survival ducks up to 40-week-old. The fertility data were also collected at 30 weeks of age.

DHBV DNA assay. Serum was used for determining DHBV DNA by spot hybridization assay using cloned DHBV DNA from Dr. P. L. Marion (1984), which was originally from Dr. Mason (Mason et al., 1982). A sample of 40 μl of serum was mixed with 200 μl STE (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0), 12 μl of 10% SDS (Sodium dodecyl sulphate), and 10 μl of proteinase K (10 mg/ml STE stock solution), digested at 37℃ for overnight. Then the DHBV was extracted with equal volume of phenol-chloroform, after centrifugation at 10,000 rpm for 5 min., the clear supernatant was denatured with 1V of 1N NaOH, vortex and held at room temperature for 10 min. The equal volume of neutralization solution (Tris 1 M, NaCl 1.5 M) was then added, 250 μl of extracted sample were applied to nitrocellulose filter which were previously soaked in 20X SSC (standard saline citrate) by a dot hybridization apparatus. The nitrocellulose filter was dried and kept at 80℃ under a vacuum for 2 hrs, prehybridized at 42℃ for 3 h, and then hybridized at 42℃ for 15 to 21 h using 5×10^5 cpm/ml of ^{32}P-labeled DHBV DNA probe, 6X SSC, 0.5% SDS, 0.01 M EDTA, 5X Denhart's solution and 100 μg/ml Salmon sperm DNA. The filter was then washed twice for 15 min. each with 2X SSC-0.1% SDS at 25℃, then followed by washing at 56℃ twice, each 30 min., in 0.1X SSC-0.1% SDS. The dried filter was autoradiographed at −70℃ using Kodax XAR film. The results were compared with the standards of 1, 5, 10, 20, 100 pg DHBV-DNA applied on the same nitrocellulose filter.

Statistical analysis. SAS by General Linear Mean (GLM) procedure (Barr et al., 1979) was used for variance analyses for the differences of all traits.

Results

Among 289 ducks obtained from commercial duck farm, 4 males and 12 females were DHBV positive, i.e., 5.7% showed natural infection. The vertical transmission of DHBV was shown in this study. Among the 136 offspring produced by 11 dams that showed viraemia in the sera, 116 ducklings (85.3%) were infected by DHBV when the sera of ducklings were detected by spot-hybridization of DHBV DNA at 8 wk. of age. The other 20 ducklings from DHBV DNA-positive dams showed a negative viral DNA in their sera when they were bled at 8 wk. and assayed for the DHBV DNA of sera. Because artificial insemination was used to produce the offspring by reciprocal matings, some of these congenitally infected ducklings were sired by DHBV DNA-negative drakes. On the contrary, some DHBV DNA-negative ducklings were sired by DHBV DNA-positive drakes. Sexual transmission of DHBV were not obviously noticed in these results, although two congenitally infected ducklings were produced by crosses between DHBV-positive sire and DHBV-negative dam.

Forty-five ducklings from noninfected parents were artificially infected at one-day-old, 34 developed a viraemia while their sera were tested at 8-week-old. An infection rate of 75.5% was obtained. Eleven of experimentally infected ducklings were traced for their viraemia at an interval of 2 weeks till 24-week-old (table 1). Among the 11 ducklings, most of them had developed a viraemia by 2-week-old, and two ducks (Nos. 586 & 595) developed its viraemia between 2-4 weeks of age. Four ducks (Nos. 584, 591, 599, 600) developed persistent carriers throughout the 24-week observation period. The other seven ducks developed a transient viraemia. Among these recovered ducks, two ducks showed resistance as early as 6-8-week-old (Nos. 585 & 595), another duck cleared its viraemia at 20-week-old (No. 581).

An example of autoradiograph obtained from spot-hybridization technique was shown in Plate 1. It was clear that there were different concentrations of viral DNA in the sera, although those ducks were all identified as DHBV DNA-positive. Spots 14, 26, and 28 showed strongly positive with the intensities more than 100 pg, however, spot 9 showed faintly positive.

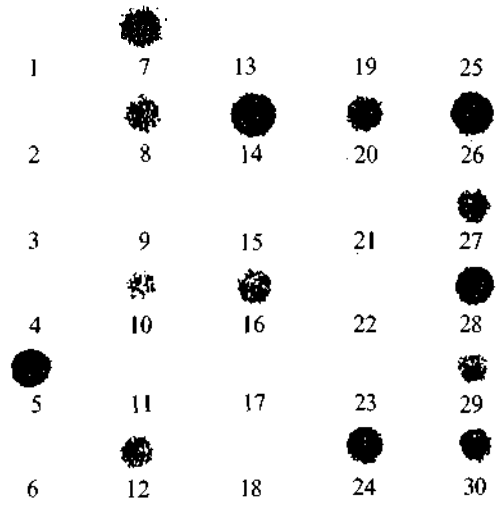

Plate 1. Autoradiography by the spot technique. Spots 1-5, positive standards of 1 pg, 5 pg, 10 pg, 20 pg, and 100 pg of DHBV-DNA respectively: spot 6, negative standard; spots 11, 13, 15, 17, 18, 19, 21-23, and 25, DHBV DNA-negative samples; other spots are DHBV DNA positive, with different amount of viral DNA in the sera. Spot 14, 26, and 28 showed strongly positive with the intensities more than 100 pg, but spot 9 is faintly positive.

TABLE 1. TRACING DHBV DNA STATUS IN THE SERA OF EXPERIMENTALLY INFECTED DUCKS[1]

Duck No.	Age											
	1-day	2 wk	4 wk	6 wk	8 wk	10 wk	12 wk	16wk	18 wk	20 wk	22 wk	24 wk
581	−	+	+	+	+	+	+	±	±	−	−	−
584	−	+	+	+	+	+	+	+	+	+	+	±
585	−	±	±	−	−	±	±	−	−	−	−	−
586	−	−	+	±	+	+	±	−	−	−	−	−
591	NT	NT	+	+	±	+	+	+	+	+	+	+
593	−	+	+	+	+	+	−	−	−	NT	−	−
594	NT	+	+	+	+	+	+	−	−	−	−	−
595	−	−	+	+	−	±	−	−	−	−	−	−
596	NT	+	+	+	+	+	+	−	−	−	−	−
599	−	+	+	+	+	±	±	±	+	+	+	+
600	−	+	+	+	+	+	+	+	±	+	±	±

[1] 11 Ducks were traced from 1-day old to 24 wk old.
± : Trace.
NT: Not tested.

Fifty DHBV-positive ducks assayed at 8 wk. of age, including 37 congenitally infected ducks and 13 experimentally infected ducks, showed no DHBV-DNA in their sera at 47 wk. of age. Thus the recovering rate from DHBV-positive to DHBV-negative was 33.3% (50/150). There was no significant difference for body weight of ducklings among three groups (table 2). The

fertility of reciprocal matings showed no significant difference among four mating types (table 3). Laying traits such as age at first egg, body weight at sexual maturity, egg weight at sexual maturity, egg weight at 45 wk. of age and body weight at 45 wk. of age, did not show significant difference for ducks among three groups. However, congenitally infected group had significantly higher egg number of survival ducks up to 40 wk. of age than that of DHBV-negative group. There was no significant difference between experimentally infected group and DHBV-negative group for egg number.

TABLE 2. EFFECT OF DHBV ON THE BODY WEIGHT OF DUCKS

Age (wk)	DHBV-DNA positive*				DHBV-DNA negative	
	Congenital infection		Experimental infection			
	BW (g)	N	BW (g)	N	BW (g)	N
Hatched	39± 4	116	40± 3	34	39± 3	126
2	174± 36	116	178± 40	34	173± 40	125
4	404±102	116	403± 91	34	388± 94	125
6	637±115	116	644±138	34	625±124	123
8	761±150	116	777±160	34	744±141	123
10	882±165	115	892±170	29	842±173	123
12	948±134	112	976±128	29	909±168	114

* The DHBV-DNA of sera were determined by spot-hybridization at 8-wk of age.

TABLE 3. EFFECT OF DHBV ON THE FERTILITY OF ARTIFICIAL INSEMINATION

Mating type (DHBV status)*	No. drake/duck	Egg-set	Fertility (%)
−/−	10/24	367	83.8
−/+	7/18	457	90.0
+/−	3/9	182	84.9
+/+	2/7	129	85.0
/−	13/33	549	80.0
/+	9/25	586	87.5
−/	17/42	824	84.5
+/	5/16	311	83.0

* The DHBV-DNA of sera were determined by spot-hybridization at 47-week of age.
/−: Pool the data from −/− and +/− mating types.
/+: Pool the data from −/+ and +/+ mating types.
−/: Pool the data from −/− and −/+ mating types.
+/: Pool the data from +/− and +/+ mating types.

Discussion

The Pekin ducks from commercial farms had a prevalence of DHBV infection of about 10-15% in the United States (Urban et al., 1985; Marion et al., 1984). Chinese common ducks (Tsaiya ducks) from commercial farms had an average positive rate of 12.2% in Taiwan (Chang et al., 1987). Because the ducks in this study were bought from one commercial duck farm only, a different infection rate (5.7%) might be expected.

The vertical transmission in this study had an infection rate of 85.3%, which was lower than the 95-100% obtained by Urban et al. (1985). Since the assay of viraemia of duck sera was conducted at 8 wk. of age, if there was any duck

which had cleared circulating DHBV by this age remained to be clarified. This has happened in the experimental infection group with a 24-week follow-up (table 1). The infection rate was affected by several factors (Dienstag, 1984), and virus concentration of inoculation was one of the factors. One persistent carrier female duck who had six DHBV DNA-negative offspring showed a lower concentration of viral DNA in its sera than the other dams. Since the antibody to DHBV surface antigen usually not detected in ducks with persistent viraemia (Vickery et al., 1989), some genetic factor other than the status of immunity might be involved in the resistance to vertical infection.

A lower infection rate, 75.5% (34/45), was found for the experimentally inoculated 1-day-old ducklings, which was lower than the percentage of vertical transmission. Similarly, a lower infection rate was reported (Mason et al., 1983) in day-old ducklings than in 15-day-embryos. As in HBV, infection in early stage of life is more likely to induce a persistent carrier (Beasley et al., 1983). Another possible fator might be due to the transfer of passive immunity from dam to its progeny (Vickery et al., 1989). Mason et al. (1983) experimentally infected 12 day-old Pekin ducklings, and found 6 of them remained to be non-infected after a tracing up to 192-day, one duck was transient viraemia, and the other 5 developed into persistent carriers. The current study showed 58.8% (20/34) ducks developed into persistent carriers after a tracing up to 47 week of age. However, a report obtained a 100% artificial infection rate after a 54-day tracing (Tagawa et al., 1985). These variation in experimental infection results, might depend on the condition of inoculation and passive immunity aforementioned.

Brown Tsaiya is one of the most prolific duck breeds in the world. The average egg number of survival ducks up to 40-week of age was 139 ± 15 (Tai et al., 1989), and the average hen-day laying rate was 86.0% in the first year (Lee et al., 1992). The laying performance of ducks in current study was compatible to those results, especially in congenitally infected group (average egg number 135 ± 4). Thus DHBV infection, no matter congenital infection or experimental inoculation did not appear to give ill effects to the economic performance of ducks in first year production. Since the fertility of individual duck showed a big variation within groups, there was no significant difference among four groups of mating types for this trait.

The occurrence of pathological change due to DHBV infection is controversial. Some researchers found that in their construct of persistent DHBV infection the ducks did not show any significant hepatitis activity histologically (Uchida et al., 1987). Later the same group gave conclusion that DHBV infection did not appear to

TABLE 4. EFFECT OF DHBV ON LAYING TRAITS

Traits	DHBV-DNA positive*		DHBV-DNA
	Cong. infe. (N)[1]	Exp. infe. (N)[2]	negative (N)
Age at first egg (d)	134 ± 2 (57)	132 ± 3 (17)	132 ± 2 (58)
B.W. at sex. mat. (g)[3]	1,269 ±15 (57)	1,259 ±27 (17)	1,282 ±18 (56)
E.W. at sex. mat. (g)[4]	43.2± 0.8(55)	44.8± 1.4(17)	41.8± 0.9(56)
E.W. at 45-wk (g)[5]	61.8± 0.7(31)	61.3± 1.3(17)	61.6± 0.5(55)
B.W. at 45-wk (g)[6]	1,169 ±18 (28)	1,252 ±44 (16)	1,211 ±16 (39)
Egg No. up to 40 wk[7]	135 ± 4[a] (28)	129 ± 9[ab] (17)	128 ± 3[b] (39)

* See table 3.

[a,b] Values within the same row with different superscript letters differ significantly ($p < 0.05$).

[1] Congenitally infected group.

[2] Experimentally infected group.

[3] Body weight at sexual maturity.

[4] Egg weight at sexual maturity.

[5] Egg weight at 45 wk of age.

[6] Body weight at 45 wk of age.

[7] Egg number up to 40 wk of age.

provoke significant hepatitis activity or advanced liver disease in the examined ducks (Uchida et al., 1988). However, the researchers of other laboratories reported that DHBV infection caused chronic hepatitis, cirrhosis, and hepatocellular carcinoma (Omata et al., 1983; Marion et al., 1984; Yokosuka et al., 1985). The variation might be induced by the different strains of ducks, subtypes of DHBV (Lambert, et al., 1991), and/or other environmental factors. In this study, a compatible performance indicated that the DHBV infection in the first year was not harmful as far as the economic traits were concerned. Just like the HBV infection in human, a chronic infection may be persistent for many years asymptomatically for most carriers. However, whether a long term infection of DHBV will affect the performance or provoke any liver disease remained to be studied.

Since the long-term purpose of this research is to breed a resistant line and a susceptible line to DHBV infection, and to provide an animal model for studying the genetic resistance to hepatitis B virus infection. Therefore, it is worth noting that in the present study and some reports (Mason et al., 1983; Vickery et al., 1989) some ducks showed the ability to clear circulating DHBV from sera. If a genetic factor is involved, ducklings which were resistant to the infection of DHBV, either congenitally of artificially, along with the duck which were able to clear the virus from the sera, provide the protential candidates for the selection on DHBV-resistant lines. On the contrary, if they were susceptible to viral infection even in a minor challenge, a susceptible line can be started from these population.

In conclusion, the persistent infection of DHBV did not exhibit any effects on the first year performance of Tsaiya ducks. Both infection rates of vertical transmission and experimental infection which were detected by the assay of DHBV DNA in sera at 8-week-old did not reach 100% infectious rate. Whether a genetic resistance is involved needs to be clarified, and the selection for the resistant line and susceptible line may gain some insight into it.

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