

## 4. RESULTS

### 4.1 Result interpretation

#### 4.1.1 Serological tests results

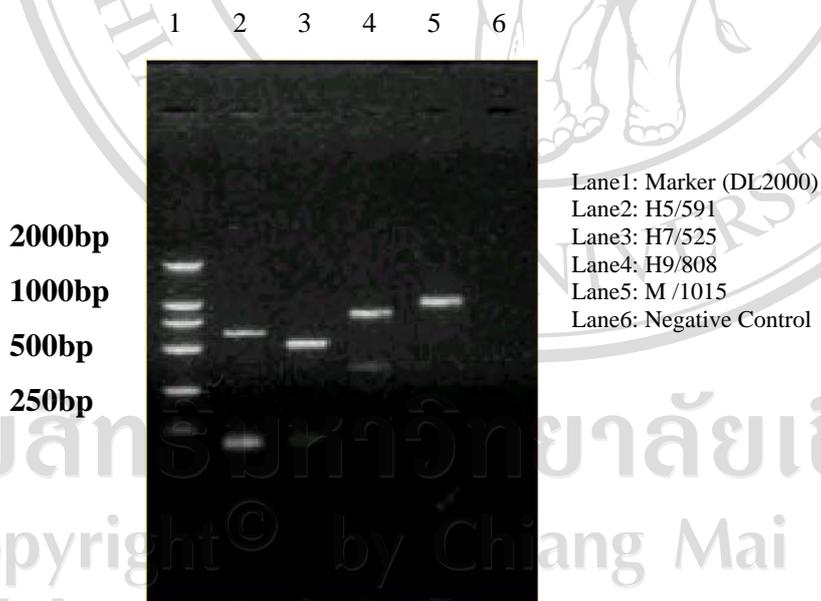
All of the tested 205 pigeons' serum samples showed negative antibody against H5 and H7 avian influenza virus. But the cumulative rate of positive antibody against H9 avian influenza virus and Newcastle Disease virus were 24.4% and 11.8% respectively (Table 4.1). Absence of the detailed information on the immunization background of the pigeons does not allow the in-depth analysis on why the population presented so high antibody against H9 avian influenza virus and Newcastle Disease virus.

**Table 4.1 The serological tests positive results statistics**

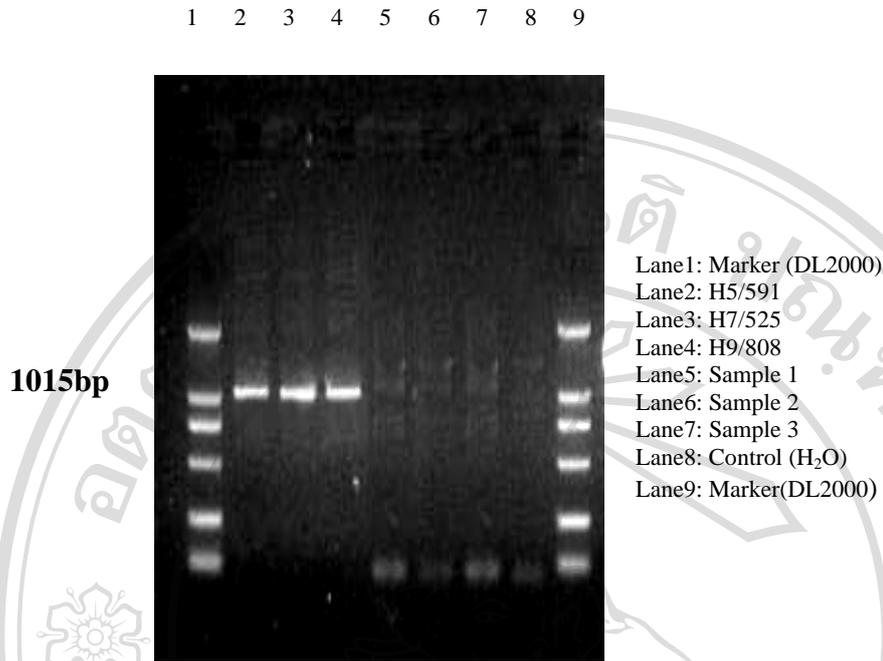
Antibody Titre (lg2)	Positive rates	
	H9 avian influenza virus (%)	New Castle Disease (%)
3	11.2	5.9
4	7.3	3.9
5	4.9	2.0
6	1.0	0
Cumulative rates	24.4	11.8

#### 4.1.2 Virus detection and virus isolation of AIV

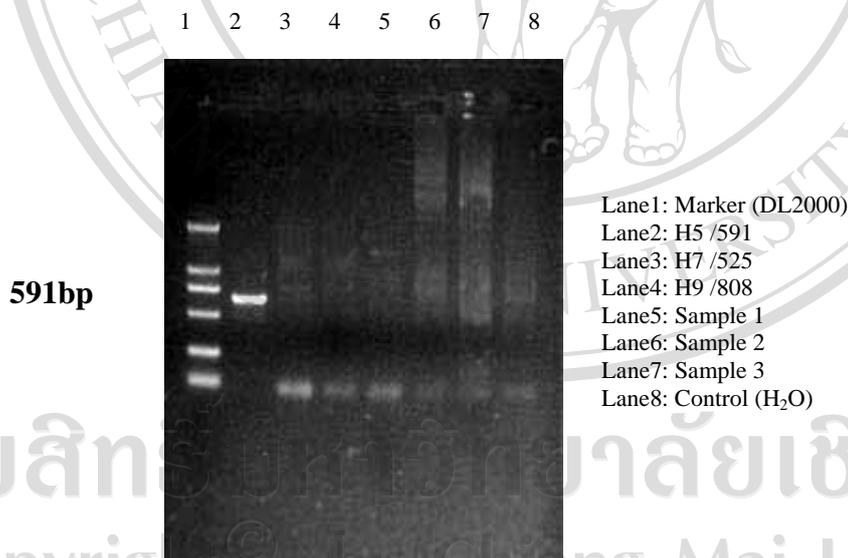
All the swabs collected from the 205 pigeons showed negative results for both the virus detection by RT-PCR and virus isolation by SPF eggs cultivation. 3 reference strain referred in 3.5.2.1 which was originated from Veterinary Reference Lab of United Kingdom and kept by Harbin Veterinary Research Institute, were used as amplified target to be positive controls (Figure 4.1- 4.5). The corresponding cDNA fragment can be amplified from the 3 reference strains of H5, H7 and H9 avian influenza virus by 3 pairs of primers H5/591, H7/525 and H9/808 (Figure 4.1). The corresponding cDNA fragment can be also amplified from the 3 reference strains of H5, H7 and H9 avian influenza virus by M/1015(Figure 4.2). These indicate that the reverse transcription procedure and the PCR protocols are appropriate. Samples in this study as Sample 1, Sample 2 and Sample 3 presented negative results by RT-PCR (Figure 4.3, Figure 4.4, and Figure 4.5) and the specificity of each primer were also presented.



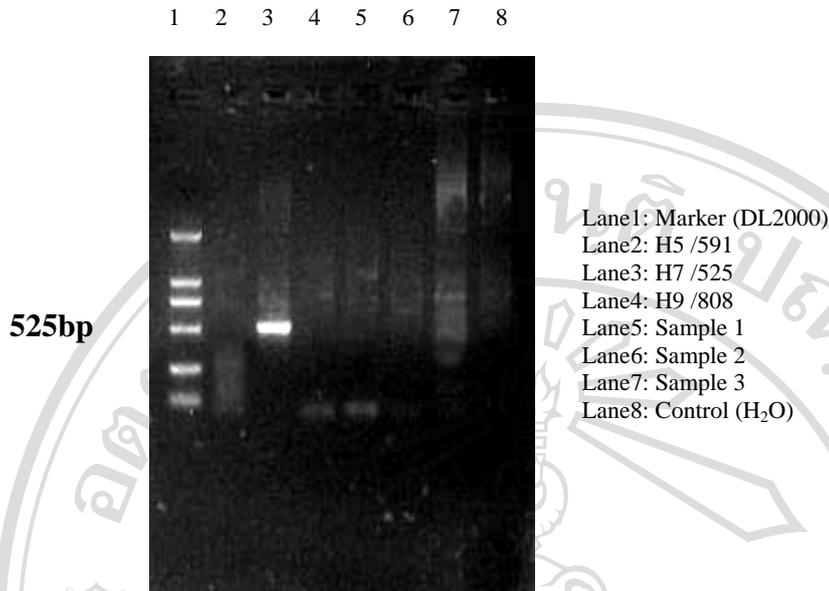
**Figure 4.1** Fragment amplified from 3 pairs of primers H5/591, H7/525 and H9/808



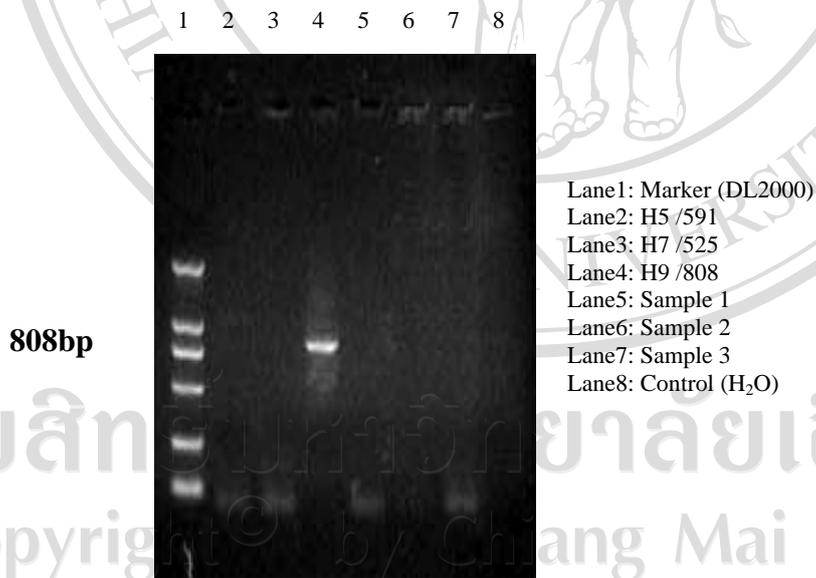
**Figure 4.2** Fragment amplified results of sample 1, 2 and 3 by M/1015



**Figure 4.3** Fragment amplified results of sample 1, 2 and 3 by H5/591



**Figure 4.4** Fragment amplified results of sample 1, 2 and 3 by H7/525



**Figure 4.5** Fragment amplified results of sample 1, 2 and 3 by H9/808

## 4.2 Alternative explanation

### 4.2.1 Maximum probable prevalence

According to Win Epicope 2.0, the following formula was used to estimate the maximal number of positive (D) given that all the 205 pigeons sampled from the Harbin LBMs showed negative results.

$$D = [1 - (1 - CL)^{1/n}] * [N - (n - 1)/2]$$

Where:

D = the maximal number of positive pigeons

CL = confidence level as a fraction (here 0.95 was adopted)

N = population size

n = sample size in this study

The population of city pigeons in Harbin estimated presently is 200000 and the sample number was 205, the maximal number of positive pigeons would be 289. In the other word, the maximum probable prevalence among those 200000 city pigeons would be 1.45%.

Although no positive results from the 205 pigeons by virus detection and isolation, it does not mean H5N1 avian influenza virus was not present among the targeted population. The maximum probable prevalence can interpreted as following: if the prevalence was higher than 1.45%, there would be at least one positive result among 205 samples. So the real prevalence of H5N1 avian influenza virus among city pigeon in Harbin city is lower than 1.45%.

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#### 4.2.2 Selection of the live birds market

The results of all samples for virus isolation were negative, it maybe caused by the systematic selection bias. The interpretation of the negative results cannot verify that there is no infection in the pigeons in Harbin city, but it is not excluded that the pigeons hold low potential veterinary public health importance as to the transmission of the avian influenza.

#### 4.2.3 The positive antibody level against H9 avian influenza virus and New Castle Disease

The presence of detectable antibody indicates that an animal or its dam has been exposed to the antigen that stimulates the antibody's production. In the absence of further challenge, the antibody level will decline.

But in this serological survey of avian influenza virus infection, titers to some antibodies have a slow rate of decline. The possession of a long half-life explains why some vaccines can produce lifelong immunity after a single course. The half-life vaccine antibodies therefore are an important aspect of vaccines efficacy and of passively acquired immunity in young animals. But the half-life of antibodies following natural infection, however, is rarely estimated. It has been reported infections of New Castle Disease in pigeons in the previous epidemiology studies of the city pigeons. Due to the inaccessibility to the detailed background information of every retailer, it is difficult to conclude whether the antibody is from the immunization or natural infection.