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Research Article

Genomic Analysis of Endogenous Retrovirus Elements in Chinese Flocks of Economic Importance [1]

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Abstract

Since the status of endogenous retrovirus elements in Chinese chickens is currently unknown, the embryonated eggs were analyzed from 10 different chicken breeds throughout China. In this study, endogenous retrovirus elements were analyzed including: EAV, *ev, ev*/J, and ART-CH in the embryos of economically important chicken flocks in China. These results indicated that compared with the E51 and EAV-0, the EAV genomic sequence in the tested chicken breeds was more closely related to EAV-0. The ART-CH elements in Chinese chickens were not significantly different from the prototype ART-CH clones, 5 and 14. Although the nucleotide acid sequences of ev and ev/J in the tested chicken breeds was similar to other known ev and ev/J sequences, they belonged to different branches in the phylogenetic tree (except for Lohmann Brown layers and White Leghorns). In addition, the intact ev/J sequences indicated that these chickens were somewhat different from each other. The results reported here demonstrate that endogenous ALVs are widely distributed throughout a number of Chinese chicken breeds. The endogenous viral genomes present in the Chinese chicken breeds are genetically distinct from other endogenous viruses circulating within chicken populations.

Keywords: *Avian leukosis virus, Endogenous retrovirus, Chicken, Genetic divergence, Prevalence*

Çin'de Ekonomik Öneme Sahip Tavukçuluk İşletmelerinde Endojen Retrovirüs Elemanlarının Genomik Analizi

Özet

Çin tavuklarında endojen retrovirüs elemanlarının durumu henüz bilinmediğinden, Çin genelindeki 10 farklı tavuk ırkına ait embriyolu yumurta analiz edildi. Sunulan çalışmada, Çin'de ekonomik açıdan önemli tavuk sürülerinin embriyolarındaki EAV, *ev, ev*/J ve ART-CH'yi kapsayan endojen retrovirüs elemanları analiz edildi. Bu sonuçlar, test edilen tavuk ırklarındaki EAV genomik dizisinin E51 ve EAV-0 ile karşılaştırıldığında EAV-0 ile daha yakın ilişkili olduğunu gösterdi. Çin tavuklarındaki ART-CH elemanları, prototip ART-CH klonları 5 ve 14'ten önemli düzeyde farklı değildi. Test edilen tavuk ırklarındaki ev ve ev/J nükleotid asit dizileri, diğer bilinen ev ve ev/J dizilerine benzer idiyse de, filogenetik ağacın farklı dallarına aitti (Lohmann Brown yumurtacıları ve Beyaz Leghorn'lar hariç). Ek olarak, sağlam ev/J sekansları, bu tavukların birbirinden biraz farklı olduğunu gösterdi. Bildirilen sonuçlar, endojen ALV'lerin bir kaç Çin tavuk ırkı arasında yaygın şekilde dağıldığını göstermektedir. Çin tavuk ırklarında mevcut endojen viral genomlar tavuk popülasyonları içinde dolaşan diğer endojen virüslerden genetik olarak farklıdır.

Anahtar sözcükler: *Kuş leukosis virüsü, Endojen retrovirüs, Tavuk, Genetik farklılık, Prevalans*

INTRODUCTION

Avian leukosis viruses (ALV) were α-retrovirus that can be classified into 10 subgroups (A-J) based on characteristics, including host range, cross-neutralization, and envelope interference [1]. ALVs can be further divided into either exogenous or endogenous viruses based on the mechanism of transmission [2]. Exogenous viruses (subgroups A to D and J) can be spread vertically from the hen to the embryo through the egg, or horizontally from chicken to chicken [3]. ALV subgroups A, B, and J are common throughout the poultry industry; however,

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C and D are less common [2-5]. Among the endogenous viruses, four new ALV subgroups (F to I) were discovered and isolated from wild birds (e.g., pheasant and quail) [6]. ALV subgroup E (termed *ev* loci) is ubiquitous, exhibits low pathogenicity in chickens, and is the best studied endogenous virus to date [7].

Four major families of endogenous retrovirus elements: (i) Endogenous Avian Retrovirus (EAV), (ii) *ev* loci (Now termed ALV-E), (iii) avian retrotransposon from the chicken genome (ART-CH), and (iv) chicken repeat 1 (CR1) were identified in chickens [8]. The *ev* loci have been studied and characterized extensively [9]. The *ev* are inserted into the germline of normal chickens and are subsequently transmitted via Mendelian inheritance [10]. Some of the *ev* are closely related to the ALV exogenous viruses, with the exception of ALV-J [8]. The EAV consist of several different types of proviruses, including EAV-0, EAV-E51 and EAV-HP, meanwhile E51 was older than EAV-0 [11-13]. Members of the EAV family cannot form an infectious viral particle, but the RT (Reverse Transcriptase) remains functional and has been found in several human live vaccines, such as measles and mumps vaccines as chicken cells are used as a preparation method [14]. Additionally, ART-CH elements consisting of functional LTRs (Long Terminal Repeat) and short regions homologous to the ALV *gag*, *pol*, and *env* sequences can be found in around 50 genomic copies in the chicken genome [15,16]. The *gag*-related sequences are located within ART-CH elements, the longest of which is the coding sequences for p10, the matrix proteins (MA), and the capsid (CA) [16]. However, the sequences encoding the nucleocapsid (NC) and protease (PR) are not included [16]. The *ev*/J elements (Also termed EAV-HP) are novel sequences contained within the chicken genome that are highly similar to the HPRS-103 *env* gene of the ALV-J subgroup [8]. The ALV-J *env* was believed to have originated from the *ev*/J as it shares 95% identity to *ev*/J [17,18]. The CR1 element was a short, interspersed repetitive DNA section that belonged to the non-long terminal repeated (LTR) retrotransposons obtained by RT sequences. The majority of these sections contain between 7.000 to 20.000 repeats and are likely conserved ancient sequences, preceding aves evolution, but are not functional [3].

The emergence of ALV-J is thought to have derived from a recombination event between exogenous ALVs and the endogenous retrovirus *ev*/J [18-20]. The *ev*/J *env* gene is considered to be the source of the *env* gene found in all ALV-J viruses. The enhanced susceptibility of cancerous growths arising from the ALV-J virus varies among the various chicken genetic lines, but layers have a lower tendency to form tumors [6,10]. Therefore, it is extremely important to investigate the status of infections with avian endogenous retrovirus in Chinese chickens to elucidate the relationship between the prevalence exogenous and endogenous ALVs. This research will extend our insight into the characteristics of endogenous retrovirus elements in Chinese chicken flocks of economic importance. In addition, this information will aid in the development and implementation of measures that will help reduce exogenous and endogenous ALV infection prevalence in Chinese poultry. This study investigated the status of endogenous retrovirus elements in embryonated egg from seven breeds of indigenous Chinese chickens and three kinds of adventitious chickens raised widely throughout China using virus-specific PCR assays.

MATERIAL and METHODS

Samples

Fertile eggs were examined from ten different chicken breeds: 1) Shouguang chicken, 2) Beijing fatty chicken, 3) Langshan chicken, 4) Taihe chicken, 5) Pudong chicken, 6) Suqin chicken, and 7) Green eggshell chicken (All these embryos were from preserving species field in China) which are the important indigenous locks of economic importance in China, as well as 8) Lohmann Brown layer, 9) White Leghorn, and 10) Ross Brown layer. The adventitious chicken embryos were purchased from the chicken breeding companies in China. The DNA was extracted from the chicken embryo fibroblasts (CEFs) from 10-d-old embryonated chicken eggs obtained as described elsewhere [9], using with mincing and trypsin treatment.

DNA Extraction

DNA extraction was performed using a Genomic DNA Extraction Kit (Shanghai Generay Biotech. Co. Ltd., Shanghai, China). The genomic DNA of the CEFs of the tested chickens was resuspended in 50 µL of DNase-free water and was stored at -80°C.

Polymerase Chain Reaction (PCR)

The PCR assays used for detecting and sequencing the endogenous retrovirus elements in the CEFs were performed using the specific primer sets for each endogenous ALV *(Table 1)*. The reaction volume was 25 µL, which consisted of 1 µL of the template, 2.5 µL of 10 × buffer (Mg^{2+} free), 2 µL of $MgCl_2$ (25 mM), 2 µL of dNTP mixture (2.5 mM each), 1 µL of the forward primer (25 pmol), 1 µL of the reverse primer (25 pmol), 0.25 µL of Lataq polymerase (Takara Biotechnology Dalian Co. Ltd., Dalian, China), and the appropriate volume of DNase-free distilled water. The PCR procedure was as follows: preheat for 3 min at 94°C, 33 cycles of 40 s at 94°C, 40 s at the required temperature for each primer pair *(Table 1)*, extension at 72°C (according to the size of the fragments, 1 kb/min), and a final extension at 72°C for 10 min. 1.0% agarose gel electrophoresis was used to evaluate the PCR products.

Sequencing of DNA Products

Endogenous retrovirus genetic diversity was evaluated via nucleotide (nt) and amino acid (aa) sequences derived from a single 10-day-old embryonated egg from each

chicken breed. The *env* gene was used to design the primer pairs for the sequencing the *ev* and *ev*/J genomes due to the hyper variation of the sequences for nt and aa. The primer pair for detecting the intact *ev*/J genome sequence and that designed to allow for the specific amplification of proviruses containing complete *pol* genes were both set. The primer pair used to sequence the EAV genome was obtained from an area between the transmembrane (TM)-coding domain of the *env* and the long terminal repeats. This is because a large deletion occurred in the location of the EAV-0 *env* surface -coding domain. The sequencing primer pair for the ART-CHs was derived from the ART-CH *gag*-related sequences since the ART-CH internal regions were completely defective.

Table 1. *Oligonucleotide primers*

Family	Target Gene	Sequence 5′→3′	Annealing Temp. (°C)	Products Size (bp)	References
EAV	TM and LTR	F:gatgtgaggatgtcgaagg R:acaaagcatggaagacaga	46	241	[2,22]
ART-CH	*gag*- related region	F:ctcaaggtggctcatttaac R:acaaagcatggaagacaga	46	657	[2]
ev loci	*env*	F:ggatgaggtgactaagaaag R:tttgactgtctgcacatctc	48.5	881	[2,18]
		F:caatccttttctttaacagcg R:taacggaccaacaggctagt	46.5	713	[2]
ev/J	*env*	F:acaccattggtggcgcgtgtc R:cccgtcacatcgcgttc	48.5	1480	[9]
	Intact gene	F:ttcgtgattggaggaaacacttg R:gttacacttggcacacaaaggtggcataac	60	3900	[9]
	pol	F:ttcgtgattggaggaaacacttg R:cacgtttcctggttgttg	50	568	[15]

F: *forward primer,* ***R:*** *reverse primer*

Table 2. *GenBank accession numbers of the Chinese and the reference ALVs used in the phylogenetic analysis*

Subgroup/ Family	Name	Accession Number	Subgroup / Family	Name	Accession Number
A	RSA	M37980	*ev*-J	Line N chicken	NC_005947
B	RAV-2	M14902		Line 21 chicken	AJ238125
C	Prague C	V01197		Red jungle fowl	AJ238121
D	Schmidt-Ruppin D	D10652		Grey jungle fowl	AJ238122
E/ART-CH	clone 5	L25261		*ev*/J-BF	KU504577
	clone 14	L25262		*ev*/J-GS	KU504578
	ART-CH-BF	KR188978		*ev*/J-LB	KU504579
	ART-CH-GS	KR188979		*ev*/J-LS	KU504580
	ART-CH-LB	KR188980		*ev*/J-PD	KU504581
	ART-CH-LS	KR188981		*ev*/J-RB	KU504582
	ART-CH-PD	KR188982		*ev*/J-SG	KU504583
	ART-CH-RB	KR188983		*ev*/J-SQ	KU504584
	ART-CH-SG	KR188984		*ev*/J-TH	KU504585
	ART-CH-TH	KR188985		*ev*/J-WH	KU504586
	ART-CH-WL	KR188986	E/*ev* loci	*ev*-1	AY013303
	ART-CH-SQ	KR188987		*ev*-3	AY013304
E/EAV	EAV-0	X59844		*ev*-6	AY013305
	E51	M95189		*ev*-BF	KR188998
	EAV-BF	KR188988		*ev*-GS	KR188999
	EAV-GS	KR188989		ev-LB	KR189000
	EAV-LB	KR188990		*ev*-LS	KR189001
	EAV-LS	KR188991		*ev*-PD	KR189002
	EAV-PD	KR188992		*ev*-RB	KR189003
	EAV-RB	KR188993		*ev*-SG	KR189004
	EAV-SG	KR188994		*ev*-SQ	KR189005
	EAV-SQ	KR188995		*ev*-TH	KR189006
	EAV-TH	KR188996		*ev*-WL	KR189007
	EAV-WL	KR188997	J	HPRS-103	Z46390

The PCR products were purified using a Gel Purification kit (Beijing Dingguo Changsheng Biotechnology Co. Ltd., Beijing, China) according to the manufacturer's instructions and then cloned into a pMD-19T simple vector (Takara Biotechnology Dalian Co. Ltd., Dalian, China). The ligation products were transformed into DH5α and tested by bacterial fluid PCR (Beijing Dingguo Changsheng Biotechnology Co. Ltd.), then the positive samples were sequenced by Sanger method (Tsingke Biological Technology Co. Ltd. Wuhan, China). Sequences of the EAV, *ev*, *ev*/J, and ART-CH were retrieved from the GenBank database *(Table 2)*. Aligning and phylogenetic analyzes were performed using DNAStar (DNAStar, Inc., Madison, WI).

RESULTS

EAV, *ev*, *ev*/J, and ART-CH are the known endogenous retrovirus elements. The PCR assays that utilized specific primers for each endogenous retrovirus revealed that all of the retrovirus elements are present in the CEFs in all of the chicken breeds that were examined. An nt sequence comparison of the TM genome and the EAV LTR detected in embryonated eggs from all of the tested chickens showed a 95.9% - 100% sequence identity and between a 96.3% - 99.6% identity with EAV-0. However, E51 showed only 73.0% - 76.3% identity to the EAVs from the 10 chickens investigated in this study. The deduced aa sequences of the EAV detected in all the chickens showed 92.5% - 100% identity to each other, 93.8% - 98.8% identity to EAV-0, and 50.0% - 53.8% identity to E51. These results showed that the tested chicken EAVs are closely related to EAV-0, but distantly related to E51. The phylogenetic data derived from the total number of nt substitutions also found similar results *(Fig. 1)*.

To investigate the ART-CH prevalence and its genetic diversity in Chinese, as well as other chicken breeds, this study investigated the variability in the ART-CH, nt sequence since it does not encode aa as a result of large multiple nt deletions. Paired comparisons of the *gag*-related nt sequences revealed that the ART-CHs detected in the tested chickens had a sequence identity of 91.8% - 98.3% to each other but a higher sequence identity to prototype strains, the ART-CH clones 5 (93.5% - 99.2%) and 14 (92.1% - 99.1%). From the total number of nt substitutions and deletions, the *gag*-related sequence phylogenetic tree was analyzed using the prototype ART-CH elements, clones 5 and 14. It was found that the *gag*-related nt ART-CHs sequences showed that PD, TH, and BF were in a different branch of the phylogenetic tree compared with others. Moreover, LB and RB is closely related to the prototype ART-CHs clones 5 and 14 *(Fig. 2)*.

The nt sequence of the *ev* genomes (a part of the *env* gene) paired comparisons of the nt sequence substitutions denoted the maximum sequence divergence between the *ev* loci in the CEF of Ross Brown, and prototype *ev*-6, even for small substitutions (1.4%). Such nt substitutions only resulted in 4.2% of the aa alterations. In general, the nt and aa sequences for a region of the *env* gene from the *ev* genomes revealed a high level of identity. The nt sequence

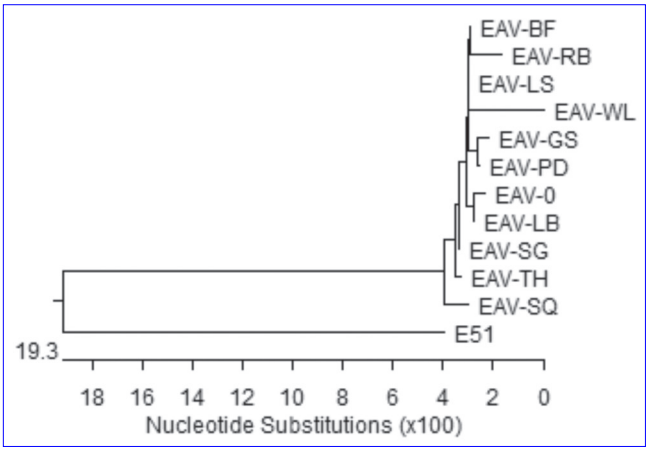

Fig 1. Phylogenetic tree of the nucleotide sequences from the endogenous EAV genomes made using MegAlign (DNAStar)
EAV-BF: Beijing fatty chicken; EAV-RB: Ross Brown layers; EAV-LS: Langshan chicken; EAV-WL: White Leghorn; EAV-GS: Green eggshell chicken; EAV-PD: Pudong chicken; EAV-LB: Lohmann Brown layers; EAV-SG: Shouguang chicken; EAV-TH: Taihe chicken; EAV-SQ: Suqin chicken; and EAV-0 and E51: Prototype of EAV

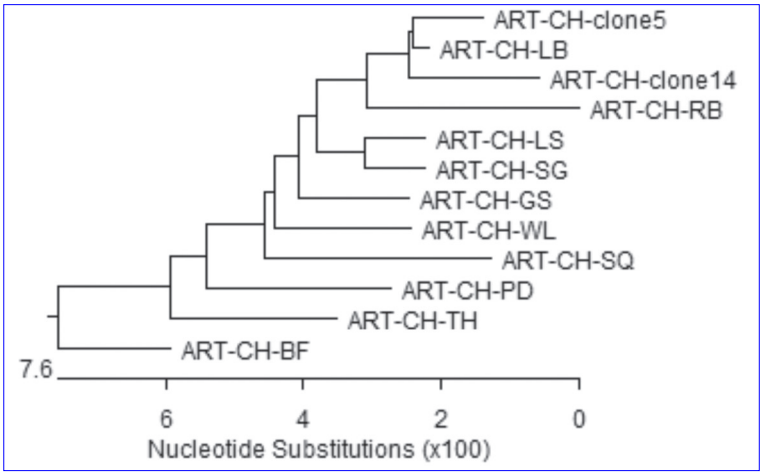

Fig 2. Phylogenetic tree of the nucleotide sequences from the endogenous ART-CH element made using MegAlign (DNAStar)
ART-CH-BF: Beijing fatty chicken; ART-CH-RB: Ross Brown layers; ART-CH-LS: Langshan chicken; ART-CH-WL: White Leghorn; ART-CH-GS: Green eggshell chicken; ART-CH-PD: Pudong chicken; ART-CH-LB: Lohmann Brown layers; ART-CH-SG: Shouguang chicken; ART-CH-TH: Taihe chicken; ART-CH-SQ: Suqin chicken; and ARTCH-clones 5 and 14: Prototype of ART-CH

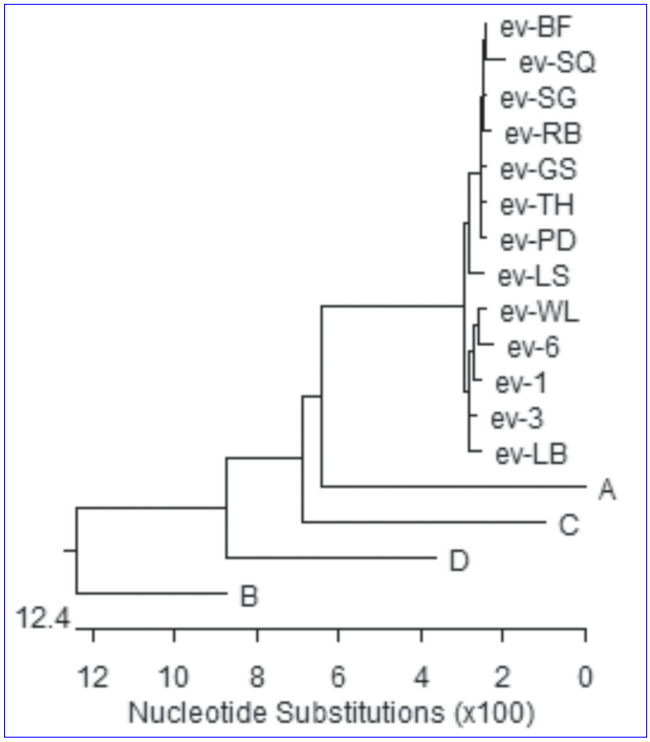

Fig 3. Phylogenetic tree of the nucleotide sequences from the endogenous ev genomes made using MegAlign (DNAStar)
ev-BF: Beijing fatty chicken; *ev*-RB: Ross Brown layers; *ev*-LS: Langshan chicken; *ev*-WL: White Leghorn; *ev*-GS: Green eggshell chicken; *ev*-PD: Pudong chicken; *ev*-LB: Lohmann Brown layers; *ev*-SG: Shouguang chicken; *ev*-TH: Taihe chicken; *ev*-SQ: Suqin chicken; and A, B, C and D: subgroup A, B, C and D ALSVs

between *ev* genomes and ALV-A was the most closely related one, while that with ALV-B was the most distant *(Fig. 3)*.

A section of the nt and aa sequences of the *env* gene from the *ev*/J genome demonstrated high sequence identity (nt; 95.9% - 99.1% and aa; 96% - 99.8%) among all of the tested chickens, as well as the other known sequences of *ev*/J. Moreover, all of the tested chickens contained the *ev*/J genomes, and the other known *ev*/J revealed a 92.8% - 98.1% nt and 96.1% - 98.2% aa identity to ALV-J. Phylogenetically, all of the compared *ev*/J genomes are randomly distributed, and the genetic distance of the *ev*/J genomes and ALV-J was relatively close *(Fig. 4)*.

Results of the PCR assay for detecting the complete *ev*/J genome were quite different between samples. Products of approximately 3.9 kb were amplified with the genome DNA template from all of the tested chickens. However, additional products of approximately 2.1 kb were amplified with Shouguang chickens, Beijing fatty chickens, Langshan chickens, and three adventitious breeds *(Fig. 5)*.

The 568 bp product was amplified with all the genomic DNA templates from all of the tested chickens in the test for detection of the *pol* gene in *ev*/J, with the exception of Taihe and Pudong chickens. These results illustrated that the complete *ev*/J genome differed between chicken breeds and none of the *ev*/J *pol* gene was intact in the tested chickens *(Fig. 6)*.

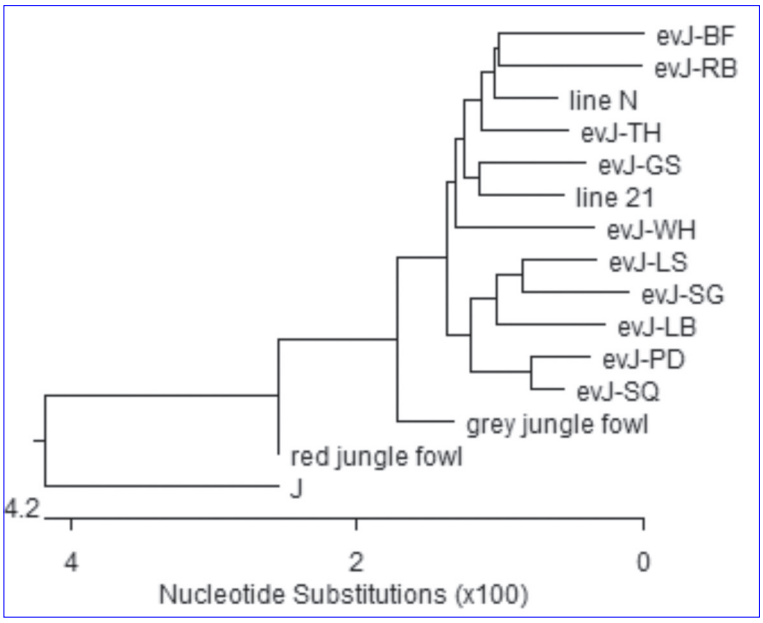

Fig 4. Phylogenetic tree of the nucleotide sequences from the endogenous ev/J genomes made using MegAlign (DNAStar).
ev/J-BF: Beijing fatty chicken; *ev*/J-RB: Ross Brown layers; *ev*/J-LS: Langshan chicken; *ev*/J-WH: White Leghorn; *ev*/J-GS: Green eggshell chicken; *ev*/J-PD: Pudong chicken; *ev*/J-LB: Lohmann Brown layers; *ev*/J-SG: Shouguang chicken; *ev*/J-TH: Taihe chicken; *ev*/J-SQ: Suqin chicken; and J: subgroup J ALSVs. Green jungle fowl, red jungle fowl, line 21 and line N: *ev*/J

phylogenetic tree from the *env* gene showed that the *ev* genomes of the tested chickens were highly related. However, the *ev* genomes, including prototype *ev*-1, *ev*-3, and *ev*-6 were separately clustered with the exception of the Lohmann Brown layers and White Leghorns. Besides, among the exogenous viruses, the genetic distance

DISCUSSION

Sequence analysis of the location between the TM and the LTR of the tested EAVs indicated that there is a low sequence identity to E51 and a high sequence identity with the other EAVs, including EAV-0 and the other tested

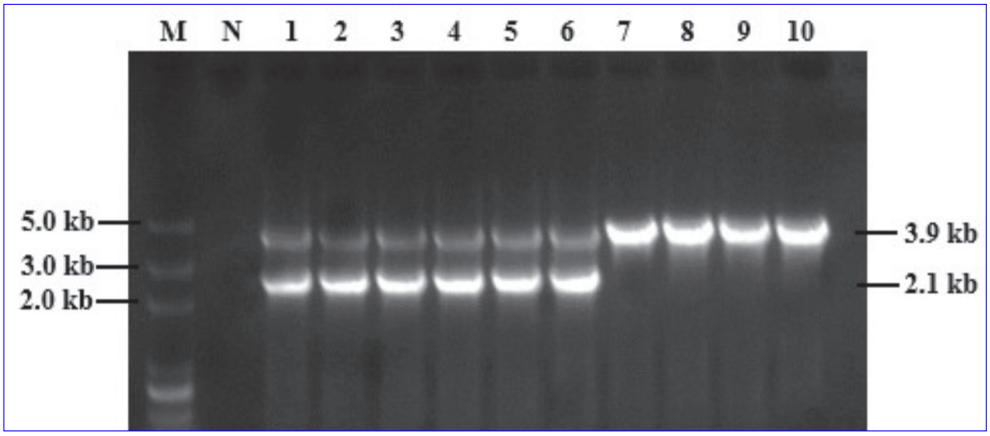

Fig 5. Reaction products of intact endogenous *ev*/J genomes subjected to electrophoresis on a 1% agarose gel.
Lane 1: Beijing fatty chicken; lane 2: Langshan chicken; lane 3: Lohmann brown layers; lane 4: Ross brown layers; lane 5: Shouguang chicken; lane 6: White Leghorns; lane 7: Green eggshell chicken; lane 8: Pudong chicken; lane 9: Suqin chicken; lane 10: Taihe chicken; N: Negative control (no template control); and M: DNA marker

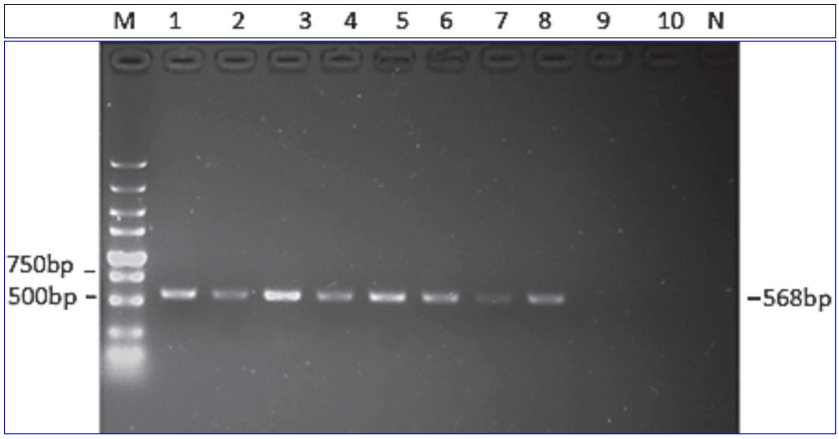

Fig 6. Reaction products of the selective amplification of *ev*/J proviruses with intact pol genes subjected to electrophoresis on a 1% agarose gel
Lane 1: Beijing fatty chicken; lane 2: Langshan chicken; lane 3: Green eggshell chicken; lane 4: Lohmann Brown layers; lane 5: Ross Brown layers; lane 6: Shouguang chicken; lane 7: Suqin chicken; lane 8: White Leghorn; lane 9: Pudong chicken; lane 10: Taihe chicken; N: Negative control (no template control); and M: DNA Marker

EAVs. The EAV is present in all Gallus species, consistent with a germline infection occurring before speciation. In addition, analysis of the EAV family revealed that the Gallus species genomes are heterogeneous. This is likely the result of prolonged evolutionary pressure compared to other endogenous retroviruses [21]. Correspondingly, different EAV family members might have infected the Gallus species throughout its evolutionary history. For example, EAV-0 may be younger than E51. Our results demonstrated all of the tested EAV underwent a pathway similar to EAV-0, but different from E51.

The evolutionary relationship between the various ART-CH and the other endogenous retroviruses remains unclear. A comparison of a section of the *gag*-related gene sequences observed in the ART-CH of the tested chickens with that of the other known ART-CH implies that they are distinct from each other (91.8% - 98.3% sequence identity). However, they are closely related to the ART-CH clones 14 (92.1% - 99.1% sequence identity) and 5 (93.5% - 99.2% sequence identity). The phylogenetic tree developed in this study also supports this claim.

In this study, the sequence analysis of an *env* gene section located between the *ev* of the tested chickens and other known *ev* genomes demonstrated a high degree of identity. Although phylogenetically their ev genomes cluster together, the prototypical *ev* genomes are clustered on a separate branch. The exception is the Lohmann Brown layers (in the same branch as *ev*-3) and the White Leghorns (in the same branch as *ev*-6), which indicates that the *ev* genomes discovered in the Chinese indigenous chickens had a differential evolutionary pathway from the prototype viruses, but that some adventitious breeds shared the same evolutionary pathway. Among the known exogenous ALVs, the *ev* genome (ALV-E) of tested chickens were closely related to ALV-A, which indicated that the ancestors of ALV-E and ALV-A were possibly similar with

each other because of the short genetic distance. Moreover, a sequences analysis of the *ev*/J genome *env* gene from the tested chickens exhibited both a high nt and aa identity in comparison to the other known endogenous genomes and exogenous ALV-J prototype strain HPRS-103. Phylogenetically, the tested *ev*/J genomes were distributed randomly among the other known *ev*/J genomes and the ALV-J prototype strain HPRS-103. However, *ev*/J genomes of the tested chickens and the prototype ALV-J strain were clustered in the same evolutionary branch indicating that they shared a common ancestor. It is well-known that the *ev*/J genomes are the material basis of the ALV-J *env* gene. However, there was no obvious nt substitution divergence observed between the *ev*/J and subgroup J ALV. Unlike gray jungle fowl, in which the *pol* gene was complete in the *ev*/J genome, none of the tested *ev*/J genomes exhibited a complete *pol* gene. Furthermore, the complete *ev*/J genome of the tested chickens was somewhat different from each other for obtaining either one or two fragments.

In this study, the primer pairs specific to the endogenous genomes of EAV, *ev*, *ev*/J, and ART-CH confirmed that these endogenous avian retrovirus elements are present in all seven types of indigenous Chinese chickens and three adventitious breeds via PCR. The results demonstrated that the endogenous retroviruses were closely related to each other in these ten chicken species, especially in indigenous Chinese chickens. Moreover, although none of the tested chickens had a complete *pol*, the genome of the *ev*/J was quite different between the Chinese indigenous chickens and the adventitious breeds.

The expression of particular endogenous proviruses may have an effect on the phenotype of the organism by influencing the susceptibility to related retroviruses, variations in the immune status, or creating genomic instability via recombination with sequences from other cells of retroviruses [22]. Additionally, the existence of different endogenous retroviruses in the various types of chicken breeds may have resulted in the broilers to have a greater probability of infection with ALV-J than the layers. Therefore, further study is required to elucidate whether other Chinese endogenous retroviruses contain similar traits as the endogenous viruses in Chinese indigenous chickens possessing these characteristics.

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