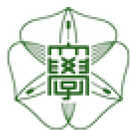
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JJVR

REGULAR PAPER **Experimental Research**

Studies on the pathogenicity of duck tembusu virus strain KPS54A61 using mice and chickens

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Abstract

In Thailand, Flavivirus strain TMUV-KPS54A61 was isolated from mosquitoes and ducks. The pathogenicity of TMUV-KPS54A61 was tested in mice and chickens. The $TCID_{50}$ of TMUV-KPS54A61 was estimated and a dilute was applied to groups A-D of adult chickens (10^5, 10^4, 10^3, 10^2 $TCID_{50}$) and BALB/c mice using intracerebral inoculation. Young chickens were inoculated with $10^7 TCID_{50}$ of TMUV-KPS54A61. Adult chickens did not exhibit the clinical signs, while organ samples tested negative by RT-PCR for the genome of TMUV. On the other hand, groups A and B of BALB/c mice and young chickens showed clinical signs including anorexia, hunched posture, fluffy hair, diarrhea and retarded growth. Pathological changes observed including perivascular cuffing, multiple clusters of gliosis in cerebral and cerebellar. Necrosis of the liver cells and interstitial nephritis in the kidney were also found in young chickens, while the spleen and pancreas are unclear the pathological changes. Immunohistochemical staining of mouse spinal cord samples was positive for the virus protein. TMUV - KPS54A61 was detected in the serum, brain, liver, kidney, adrenal gland, pancreas and spinal cord by RT-PCR. Vero cells exhibited CPE after inoculation by the virus, which was isolated from the brain, spinal cord and kidneys. TMUV-KPS54A61 could maintain itself for a prolonged time in the brain, spinal cord and liver; therefore, it could be the target organs of virus and the TMUV - KPS54A61 could be pathogenic in young chickens and BALB/c mice.

Key Words: BALB/c mice, Chickens, Duck Tembusu virus, Flavivirus, Pathogenicity

Introduction

Duck Tembusu virus (TMUV) belongs to the Genus Flavivirus of the family *flaviviridae*. With the capacity to initiate a number of illnesses in mammalian and avian species, Flaviviruses are positive single-strand RNA viruses that are typically spread via mosquitoes and ticks[11]. The viruses carried by mosquitoes can be organized into 7 subgroups comprising

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Aroa, Dengue, Japanese encephalomyelitis, Kokobera, Spondweni, Yellow fever and Ntaya[6]. The Bagasa virus, Ilheus virus, Israel turkey menigoencephalitis, Sitiawan virus, Baiyangdian virus and the Tembusu virus comprise some of the instances of the Ntaya group[12].

A Flavivirus termed the Baiyangdian virus (BYD), which has a genome that is closely related to TMUV and exhibits neurological symptoms, was detected in egg-laying and meat-type ducks in China in 2010[21]. Various laying and breeder ducks in China were found to be affected by TMUV between June and November 2010[1]. In South east China, the TMUV strain Fenfxian 2012 was also set apart from ducks[25]. Further, geese and house sparrows were some of the other avian species revealed to be diseased by TMUV[5,13,18,24]. Likewise, found in geese in China was the TMUV strain GH-2[27].

In China, chicken and geese farms were confronted by a severe drop in egg production in 2011 that was triggered by TMUV infection[13]. Further, an antibody against TMUV was uncovered in the duck farm workforces in the impacted farms in Shandong China. Intimately approximating duck TMUV strains, RNA was discovered as well[24]. A number of examiners in Thailand isolated TMUV strain KPS54A61 from mosquitoes between 2010 and 2013 (*Culex* spp.), including from meat-type, egg-laying and free-grazing ducks. Paralysis, head trembling and decreased egg production were some of the effects of infection in ducks[2,20]. Indicating a decidedly neurovirulent character as well as the ability to reproduce in the visceral organs of BALB/c mice, the strain jxsp was examined by Li et al. (2013)[16]. Besides, hosts including Kunming mice may also instigate infection with other strains of TMUV such as TMUV - SDSG[25]. Numerous strains of TMUV have a trait that can generate infection in various avian and mammalian species, as indicated by the findings[2,20,21,24,26,27]. Thus, chickens and BALB/c mice were utilized for initial trial study as avian and mammalian models to examine the features of the KPS54A61 strain. Pathogenicity experimentation is possible since SPF chickens and BALB/c mice exist in Thailand. Additionally, chicken farms in Thailand remain at risk for TMUV infection, particularly among the household chicken farms, caged layer farms in open sheds, and chickens grown near fish ponds because they possess potentially inadequate biosecurity and mosquitoes management. The objective of this research was to study the pathogenicity of the duck TMUV KPS54A61 strain utilizing chickens and BALB/c mice as avian and mammalian models for enhanced understanding of this virus's disease mechanism.

Materials and Methods

Cell and virus preparation: Vero cells and BHK-21 cells were obtained from the Faculty of Veterinary Science, Mahidol University. Vero cells were maintained in growth media (GM) containing Iscove' Modified Dulbecco's Medium (IMDM) (HyClone®, GE Healthcare, USA), 10% Fetal Bovine Serum (FBS) (HyClone®, Thermo scientific, UT, USA). BHK-21 cells were maintained in Minimum Essential Medium (MEM) (Gibco™, Invitrogen Corporation, CA, USA) and 10% FBS. Both cells were cultured in six-well tissue culture plates, with the cultures containing 3,000 and 5,000 cells/ml for Vero and BHK-21 cells, respectively. These culturing plates were then incubated into a 5% CO_2 incubator for confluent within 24 hr. The TMUV-KPS54A61 strain used for the present study was the same as the one used in a previous study by Chakritbudsabong (2015)[2]. The virus was adapted by propagation and sub-passaging up-to three passages for Vero and BHK-21 cells. The viruses from both cell cultures were titrated and prepared for challenging into chickens and mice.

Experimental animals: Three to eight week - old specific pathogen-free (SPF) BALB/c mice were purchased from the National Laboratory Animal Center (Mahidol University, Thailand), while SPF white Leghorn chickens (3 - 8 week old adult chickens and 5 - day old young chickens) were provided by THAI S.P.F CO., LTD. (Nakhon Nayok, Thailand). Blood samples were collected from all

animals used in the experiment as pre-serum before experimental infection was started.

Pathogenicity of TMUV-KPS54A61: TMUV-KPS54A61 was used for challenging animals in the experimental groups. One hundred and twenty-five mice were divided into five groups (A-E), with 25 mice per group. Group A was inoculated by intracerebral administration with 10^5 times the 50% tissue infectious dose ($TCID_{50}$)/30 µl/each, while groups B, C and D were inoculated with 10^4, 10^3 and 10^2 $TCID_{50}$/30µl/each, respectively. Group E was the negative control group, which was inoculated with MEM. Seventy-five adult chickens were also divided into 5 groups (A-E). Each chicken in Group A was challenged intracerebrally with 10^7 $TCID_{50}$ /30 µl. Virus titer as 10^6, 10^5 and 10^4 $TCID_{50}$/30 µl were used to challenge groups B, C and D, respectively. Group E was a negative control. In addition, 25 SPF young chickens were divided into 2 groups (test and control). Fifteen young chickens were inoculated intracerebrally with $10^7 TCID_{50}$/30 µl, while 10 other chickens were inoculated with IMDM as a negative control.

Sampling: At 1, 2, 5, 9 and 14 days post-inoculation (dpi.), 25 mice (5/group/dpi), 15 adult chickens (3/group/dpi) and 3 young chickens per dpi were euthanized and sampling was done for all groups. Before challenging and euthanasia, all mice and chickens had serum samples collected. The necropsy process was started for the examination of gross lesions and visceral organs such as brain, spinal cord, liver, spleen, kidney, adrenal gland, trachea, pancreas andurinary bladder samples were collected. All samples were separated into two parts: one was kept at -80˚C for RT-PCR and virus isolation, while the other was kept in a buffer containing 10% formaldehyde for histopathology.

Histopathology and Immunohistochemistry: The fixed tissues were routinely processed, sectioned and stained with hematoxylin and eosin (H&E). The lesions were examined under a light microscope. The standard immunohistochemistry (IHC) protocol was applied and all sections were deparaffinized and rehydrated with PBS, then blocked endogenous enzyme by 3% hydrogen peroxide solution for immunohistochemistry preparation. Proteinase K was then used for antigen retrieval and the non-specific reaction was blocked with 2.5% bovine serum albumin (BSA). The positive duck serum against TMUV was used as a primary antibody for mice and chicken tissue slides at dilution 1:1000 in a humidified chamber at 37˚C for 1 h and at 4˚C overnight. After rinsing with phosphate - buffered saline and Tween20 (PBS- T), sections were incubated with conjugated secondary antibodies using goat anti-duck IgY conjugated with horseradish peroxidase (Biosciences, USA) at room temperature in a humidified chamber for 1 h. The slides were rinsed by PBS–T and Diaminobenzidine DAB® (Marck, Singapore) and were added to the substrate for color developing. The slide sections were examined under a light microscope.

RT-PCR: RNA samples were extracted using FavorPrep™ Viral Nucleic Acid Extraction kit I (Favorgen, Taiwan). The reverse transcriptase (RT) reaction was generated by Superscript™ III First-strand synthesis system (ThermoScientific, UK) according to the manufacturer's instructions. The polymerase change reaction (PCR) amplified the NS5 gene using a pair of PCR primers: the forward primer was BYD5 5'- GCC ACG GAA TTA GCG GTT GT and the reverse primer was BYD8 5'- TAA TCC TCC ATC TCA GCG GTG TAG. The PCR was amplified using Dream Taq master mix (Thermo®, USA) according to the manufacturer's instructions following a thermal cycling initial denaturation step at 94 °C for 10 minutes. After this, the samples were subjected to 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72°C for 40 seconds. The final extension step was performed at 72 °C for 10 minutes.

Virus isolation: All organ samples were ground with phosphate buffer saline (PBS) and centrifuged at 13,500 rpm for 5 minutes. The supernatant was filtrated through filters with a porosity of 0.20 µm.

The filtrate was inoculated onto the monolayer of Vero cells and incubated in a 5% CO_2 incubator for 1 hr. After adsorption, the media and inoculum were poured out and washed 3 times with PBS. Maintenance medium (MM) containing 2% FBS was added into each well, and the plates were incubated in a 5% CO_2 incubator. The cytopathic effects (CPE) were observed daily for five days.

Ethics statement: These experiments were conducted at the Monitoring and Surveillance Center for Zoonotic Disease in Wildlife and Exotic Animals (MoZWE) of Mahidol University under biosafety level 3 (BSL – 3). Animal ethics were approved by the Faculty of Veterinary Science – Animal Care and Use Committee FVS-ACUC, Review protocol No. MUVS-2015-66.

Results

Pathogenicity of TMUV-KPS54A61 in experimental animals

Clinical signs were present in mice and young chickens. The mice from A, B and C of the experimental groups exhibited clinical signs at 5 and 6 dpi including anorexia, hunched posture and fluffy hair. Three mice from group A, 1 mouse from group B, and 1 mouse from C died at 7-9 dpi. The morbidity and mortality of infected mice was 15% and 5%, respectively. The young chickens showed retarded growth and presented with signs of diarrhea at 5 dpi and died at 7 dpi, with a 13% morbidity and 6 % mortality rate. In contrast, the morbidity and mortality rates were limited in the adult chickens (Table 1).

Gross lesions were apparent in the brain, liver, spleen, kidney, pancreas and thymus of infected mice and young chickens at 1, 2 and 5 dpi. Enlargement of the liver, spleen, thymus and kidney as well as petechial hemorrhaging in the liver and pancreas were found in group A mouse at 2 dpi and also congestion in the brain of group A mouse at 5 dpi. Hematoxylin and eosin staining in infected brain tissue of the group A mice at 5 dpi showed perivascular cuffing (Fig 1A) with gliosis in the cerebral at 5 dpi (Fig 1B). Young chickens showed necrosis of the liver cells at 14 dpi (Fig 1E). Nonsuppurative inflammation as perivascular cuffing (Fig 1D) and gliosis in cerebral was found in the infected brain tissue (Fig 1C), the kidney of young chickens showed interstitial nephritis at 14 dpi (Fig 1F). The spleens and pancreas of young chickens showed unclear pathological changes.

IHC staining of spinal cord samples in group A mice at 5 dpi showed positive viral protein in the neuron (Fig 2A).

Table 1. The results show the percentage of morbidity and mortality from infected experimental animals

Animals	Morbidity	Mortality
BALB/c mice	15/100 (15%)	5/100 (5%)
Young chicken	2/15 (13%)	1/15 (6%)
Adult chicken	0/60 (0%)	0/60 (0%)

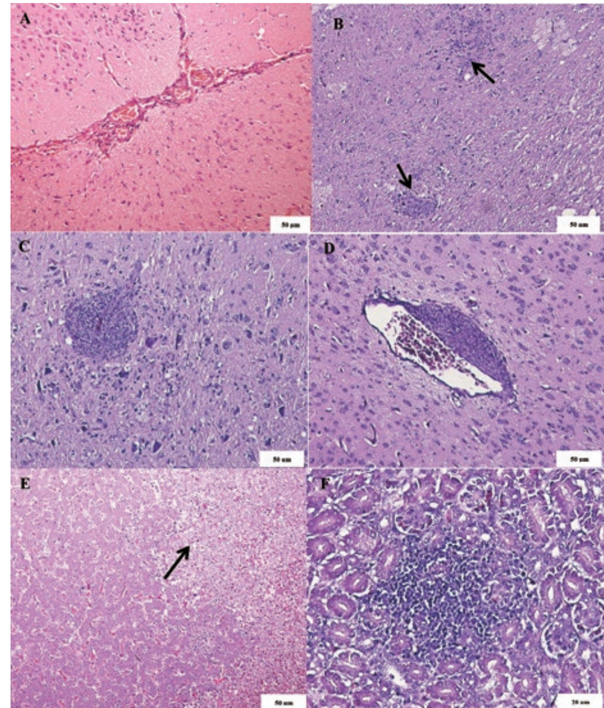

Fig. 1 Hematoxylin and eosin staining of the tissue sample. A, B: Brain tissues of group A mice at 5 dpi showed perivascular cuffing (A) and gliosis (B) and C, D: Brain tissue of young chickens at 14 dpi showed gliosis in the cerebrum (C) and perivascular cuffing (D). The liver cells of young chicken at 14 dpi were showed necrosis (E) and interstitial nephritis of the kidney (F).

Table 2. The results show the number of positive samples found by RT-PCR in young chickens' visceral organ samples after challenging with Tembusu virus titer at $10^{5.47}$ $TCID_{50}$.

dpi[a)]	Brain	Spinal cord	Liver	Kidney
1	3/3 (100%) [b)]	0/3 (0%)	3/3 (100%)	0/3 (0%)
2	2/3 (66%)	1/3 (33%)	3/3 (100%)	0/3 (0%)
5	2/3 (66%)	0/3 (0%)	3/3 (100%)	3/3 (100%)
9	1/3 (33%)	1/3 (33%)	1/3 (33%)	0/3 (0%)
14	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)

[a)] day-post-inoculation, [b)] number of positive samples out of total samples (percentage of positive samples)

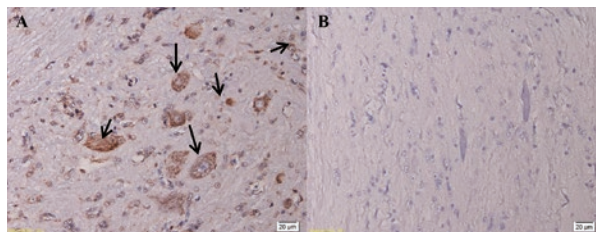

Fig. 2 Immunohistochemistry on the mouse spinal cord by using duck serum against TMUV. A, B: The spinal cord of group A mice at 5 dpi was found the target protein distribution in neuron and glial cells (A) and the negative control wasn't showing specific reaction for TMUV (B).

RT-PCR detection

The organ samples of the adult chickens tested negative for TMUV genome as detected by RT-PCR. However, the brain, spinal cord, liver, pancreas and kidney samples from the young chickens that were challenged by 10^7 $TCID_{50}$ of TMUV-KPS54A61 tested positive using RT-PCR detection. Other organ samples such as the spleen and trachea tested

negative for the TMUV genome. In detail, the brain samples tested positive by RT-PCR at 1, 2, 5 and 9 dpi at 100%, 66%, 66% and 33%, respectively. The spinal cord samples from 2 and 9 dpi were positive RT-PCR at 33% and 33%; liver samples at 1, 2, 5 and 9 dpi were positive at 100%, 100%, 100% and 33%, respectively; and 100% of kidney samples were positive at 5 dpi (Table 2). Mice were challenged by different titer doses of TMUV-KPS54A61: 10^5, 10^4, 10^3 and 10^2 $TCID_{50}$ in groups A, B, C and D, respectively. The collected samples were tested for the TMUV-KPS54A61 genome by RT-PCR. Organ samples such as serum, brain, liver, spinal cord, kidney, pancreas and adrenal gland tested positive (Table 3). Analysis of the brain samples by RT-PCR showed a positive result at 2 dpi in groups A (80%) and B (20%); at 5 dpi in groups A (80%), B (20%) and C (20%) ; at 9 dpi in groups A (40%), B (20%) and C (20%); and at 14 dpi in group A at 20%. The liver samples also showed PCR products of TMUV primers in groups A, B, C and D. The mouse liver samples tested positive at 2 dpi in groups A (80%), B (60%), C (20%) and D (20%) and at 5dpi in groups C and D (20%). The spinal cord sample of group A was demonstrated at 5, 9 and 14 dpi at 40%, 40% and 20%, respectively, and in group C at 9 and 14 dpi in both at 20%. Thekidney samples of group B also presented at 1 and 2 dpi at 20%, in group A at 2 dpi at 20%and in the pancreas samples of group A at 2 dpi at 20%. Moreover, the adrenal gland samples of group A and C were positive at 5 and 9 dpi at 40% and 20%, respectively (Table 3).

Virus isolation

Both of the infected species samples showed CPE by virus isolation. Each organ sample of each dpi was inoculated on Vero cells. TMUV-KPS54A61 was isolated from the brain and spinal cord of group A mice at 5 dpi and the kidney of young chickens at 5 dpi. The infected Vero cells exhibited syncytia and had a round formation (Fig 3A). All passages of the cultivated viruses that exhibited the CPE tested positive for the virus genome by RT-PCR.

Table 3. This table shows the results of RT–PCR testing in visceral mouse organ samples after challenging with Tembusu virus

	1 dpi[a]					2 dpi					5 dpi					9 dpi					14 dpi				
	A[b]	B[c]	C[d]	D[e]	E[f]	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Serum	-[g]	-	-	-	-	4/5[h] (80%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brain	-	-	-	-	-	4/5 (80%)	1/5 (20%)	-	-	-	4/5 (80%)	1/5 (20%)	1/5 (20%)	-	-	2/5 (40%)	1/5 (20%)	1/5 (20%)	-	-	1/5 (20%)	-	-	-	-
Liver	-	-	-	-	-	4/5 (80%)	3/5 (60%)	1/5 (20%)	1/5 (20%)	-	-	-	1/5 (20%)	1/5 (20%)	-	-	-	-	-	-	-	-	-	-	-
Spinal	-	-	-	-	-	-	-	-	-	-	2/5 (40%)	-	-	-	-	2/5 (40%)	-	1/5 (20%)	-	-	1/5 (20%)	-	1/5 (20%)	-	-
Spleen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	-	1/5 (20%)	-	-	-	1/5 (20%)	1/5 (20%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adrenal gland	-	-	-	-	-	-	-	-	-	-	2/5 (40%)	-	-	-	-	-	-	1/5 (20%)	-	-	-	-	-	-	-
Tracheal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	1/5 (20%)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]day-post-inoculation [b]Challenge with Tembusu virus titer as 10^5 $TCID_{50}$, [c]Challenge with Tembusu virus titer as 10^4 $TCID_{50}$, [d]Challenge with Tembusu virus titer as 10^3 $TCID_{50}$, [e]Challenge with Tembusu virus titer as 10^2 $TCID_{50}$, [f]non-challenge (inoculated with MEM), [g]negative result by RT-PCR, [h]The ratio of positive result samples to total samples by RT-PCR

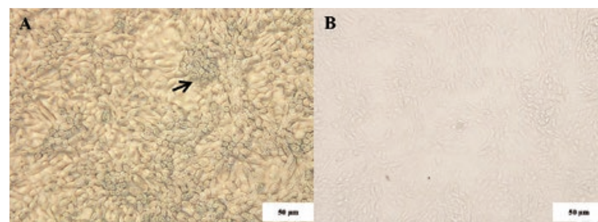

Fig. 3 Virus isolation. A, B: Infected Vero cells were exhibited syncytia and round formation (A) and non-infected Vero cells were a uniformed shape (B).

Discussion

Since 2010, TMUVs have been detected in many areas in China and isolated from ducks as well as other avian species such as geese, chicken and house sparrows[1,5,14,21,26,27]. In Thailand, TMUV was first isolated from *Culex* spp. in 1982[8]. The virus re-emerged in 2013 as TMUV-KPS54A61 when it was isolated from ducks, which exhibited nervous symptoms[2,17,20]. Many strains of TMUV have been tested and the pathogenicity of each strain isolated from different hosts or areas, which showed the variability of viral virulence. In the present study, chickens were used for pathogenesis because backyard poultry farms, which are common in Thailand, lead to contact with *Culex* spp. and are the main vector of TMUV infection. Moreover, few studies have been done on the pathogenesis of TMUV-KPS54A61 in chickens. BALB/c mice have typically been used as a mammalian model to demonstrate the pathogenesis of flaviviruses[7].

In this study, the I.C. inoculation route led to TMUV-KPS54A61 infection in chickens and BALB/c mice. Clinical signs and positive TMUV genome test results by RT – PCR (taken from organ samples) were present in young chickens and BALB/c mice but were limited in adult chickens. Clinical symptoms and pathological changes in young chickens and mice were found in groups A and B (10^5 $TCID_{50}$ and 10^4 $TCID_{50}$), indicating that these doses of the virus are enough to initiate clinical signs. This confirms previous research that found TMUVs caused severe cases of a disease in young avian species and induced clinical signs in BALB/c mice[3,18,26]. However, they do not exhibit clinical signs or pathological lesions if the infected host is an adult or infected by low doses of the virus[9,10]. This

suggests that the host age and infecting dose of the virus are important factors for infection.

TMUV – KPS54A61 infected mice demonstrated anorexia, fluffy hair, diarrhea, and hunched posture. They died at 5 - 6 dpi of the incubation period, while the young chickens exhibited fever and diarrhea, then died at 7-9 dpi of their incubation period. In mice, the range of the incubation period for other strains of the virus TMUV-SDSG[25] and TMUV-jxsp[16] in similarly aged mice is 4-6 dpi. This is not a significant difference when compared with TMUV-KPS54A61. In addition, the incubation period in young chickens is similar to previous studies when compared with layer chickens, while the incubation periods ranged from 2 dpi, 3 dpi and 6 dpi for 5-day-old ducks, 2-week-old ducks and 5-week-old ducks, respectively, which indicated that the incubation period increased in correlation with the age of the host[23]. In the present study, the findings suggest that the morbidity and mortality rates in mice are 15% and 5%, and 13% and 6% in young chickens, respectively. The morbidity and mortality rates are similar to those of Songserm's 2014 study, which reported morbidity rate ranges of 15 – 30% and mortality rates less than 10%[20]. In China, TMUV caused high morbidity with rates of 30-100%[13], 90%[22] and 100%[26]. The usual mortality rate is 3 - 30% depending on the age of the host and the viral strain of the infection[13,26]. The data in this study suggests that TMUV-KPS54A61 has a low morbidity and mortality rate when compared with the TMUVs strains in China.

The gross lesions were not found in adult chickens corresponds with a previous study in which the gross lesions were not found in adult ducks either[15]. Gross lesions were present in the brain, liver, spleen, kidney, pancreas and thymus of this study's mice and young chickens, while pathological changes were found in the brain, spinal cord, liver and kidney at 5 - 14 dpi. Particularly, a positive signal from IHC in the spinal cord of mice confirms a previous study's findings that pathological changes are usually found in the liver, lung, kidney, spleen and central nervous system[9,13,15,16,18,25]. Neuronal vacuolation in the brain was infected by TMUV-KPS54A61 strains also require to study while the neuronal vacuolation in the spinal cord was also found in Malaysia ducks TMUV[4]. Pathological changes in the brain could be seen in the form of perivascular cuffing and gliosis in the cerebral and cerebellar areas. These pathological changes in the brain and spinal cord suggest that this was the cause of the nervous symptoms. The liver also presented pathological changes such as necrosis of the liver cells, similar to findings in previous studies[13,16,25]. Mild gross lesions were also found in the spleen of young chickens at 5 dpi while the pathological changes showed unclear similar to DTMUV SDSG strain that showed varying degrees of lymphoid cell depletion[25].

Organ samples of mice such as kidney, brain, liver, spinal cord and serum tested positive for TMUV-KPS54A61 by RT-PCR. That the virus genome could be detected in the serum of mice at 2 dpi and also detected in the brain, liver, pancreas and kidney tissue suggests that this is the period of viremia after inoculation. However, it was at 5 dpi that the most number of positive organ samples were found. This was also the first day that the virus genome was found in the spinal cord of mice exhibiting clinical signs. Moreover, the virus genome remained for a prolonged period in the brain and spinal cord and could be detected by RT-PCR from 5 dpi – 14 dpi and also replicated in BHK-21 cells by virus isolation. This suggests that the brain and spinal cord of mice are the target organs of TMUV- KPS54A61. In young chickens, the virus genome was detected in the brain, spinal cord, liver and kidney at 1, 2, 5 and 9 dpi, but all organ samples were negative for TMUV-KPS54A61 at 14 dpi when tested by RT-PCR. This indicates that young chickens could be producing a neutralizing antibody that begins to seroconvert at 10 – 14 dpi. The viremia in young chickens started at 1 dpi when the virus genome in the liver was first detectable and lasted until 9 dpi. TMUV-KPS54A61 was isolated from young chicken kidneys and replicated in Vero cells. Viral doses and the prolonging of the virus in different tissues samples resulted in the production of neutralizing antibodies that began

to seroconvert[24]. The results of virus isolation indicated that TMUV – KPS54A61 could replicate in the brain and spinal cord of BALB/c mice and in the kidneys of young chickens, suggesting that TMUV has limited replication in visceral organs[10]. That TMUV-KPS54A61 could be detected for a prolonged period of time in the spinal cord, brain and liver and showed pathological change indicates that these are the target organs of the virus.

TMUV– KPS54A61 is pathogenic in chickens and BALB/c mice. This study indicates that the main target organs of TMUV– KPS54A61 are the brain, spinal cord and liver. The factors influencing TMUV pathogenesis include age, species of host, TMUV strain, virus loading and route of inoculation. These factors influence the severity of the clinical signs exhibited, the target organs, the incubation period for the replication of the virus in the infected organs and the rate of morbidity and mortality. Clinical signs were usually obvious in young avian hosts and BALB/c mice, but were subclinical in adult chickens, indicating that resistance to TMUV infection increased with age[23].

Pathogenicity testing in this study on experimental chickens and mice implies that disease prevention should focus on younger animals, which appear to be more susceptible to this virus than older animals. In field practice, there are numerous factors involved in disease susceptibilities, such as poor management and immunosuppression. This study demonstrates that TMUV-KPS54A61 can infect mammalian species such as experimental mice. This suggests that further studies should be done on the susceptibility of other mammals and on the neutralizing antibody that was found to seroconvert after infection in the experimental animals.

Acknowledgments

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