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Short Communication : Malignant Catarrhal Fever Associated with Caprine Herpesvirus-2 Infection in Captive Formosan Sika Deer (*Cervus Nippon Taiouanus*) in Taiwan

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簡訊：台灣圈飼梅花鹿感染山羊第二型疱疹病毒惡性卡他熱

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Short Communication : Malignant Catarrhal Fever Associated with Caprine Herpesvirus-2 Infection in Captive Formosan Sika Deer (*Cervus Nippon Taiouanus*) in Taiwan

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ABSTRACT Caprine herpesvirus-2 (CpHV-2) infection in Formosan sika deer (*Cervus nippon taiouanus*) with clinical signs and pathological changes of chronic malignant catarrhal fever (MCF) detected by polymerase chain reaction (PCR) and sequencing in Taiwan is reported here. During the period of September 2002 and January 2003, 12 Formosan sika deer died with progressive weight loss; while 2 other deer in the same captive herd were subclinically infected. At necropsy, gross lesions were characterized by marked arteriosclerosis in the heart, mesentery, and kidneys were noted. Microscopically, all 12 animals had severe lymphocytic vasculitis with intimal hyperplasia that were consistent with the lesions of chronic MCF. Antibody to MCF virus was detected in sera collected from all deer and goats housing in the same barn. Clinically normal goats, but not cattle, in the same barn were also PCR positive for CpHV-2. Subclinically CpHV-2-infected goats were considered to be the source of infection in this incidence. [Shih-Ping CHEN, Ming-Chung LEE, Yu-Fen SUN, Chi-Min CHEN,Ping-Chen YANG, *Ivan-Chen CHENG. Short Communication : Malignant catarrhal fever associated with caprine herpesvirus-2 infection in captive Formosan sika deer (*Cervus nippon taiouanus*) in Taiwan. Taiwan Vet J 33(3&4): 162-166, 2007. *Corresponding author TEL: (037)585-868, FAX: (037) 585-850, E-mail: icc01@mail.atit.org.tw]

Keywords: Malignant catarrhal fever (MCF), Caprine herpesvirus-2 (CpHV-2), Polymerase chain reaction (PCR)

Malignant catarrhal fever (MCF) is a serious and usually fatal infection of many species of ruminants throughout the world [9]. It is defined by the recognition of characteristic widespread lymphoproliferation, vasculitis, and inflammation affecting many tissues. Moreover, it is generally characterized by low morbidity but high mortality. Captive deer, such as red deer, Père David's deer, white-tailed deer, and sika deer, are susceptible to this disease. It has been described as one of the most important viral diseases in farm deer in New Zealand [1].

Two distinct herpesviruses, alcelaphine herpesvirus 1 (AHV-1) and ovine herpesvirus 2 (OvHV-2), are capable of inducing MCF. The AHV-1 and OvHV-2 infections are endemic in wildebeest and domestic sheep, respectively, causing virtually no clinical disease in these species. Another MCF virus that is well adapted in domestic goats has recently been identified as caprine

herpesvirus 2（CpHV-2）[4, 7, 8]. Recent studies have shown that sika deer（*Cervus nippon*）and white-tailed deer（*Odocoileus virginianus*）cohabiting with infected domestic goats can lead to the development of MCF in those deer [7, 8]. The detection of DNA of CpHV-2 from peripheral blood leukocytes from sick deer and clinically healthy domestic goats further strengthens the concept that goats are the reservoir for CpHV-2 [4, 7, 8]. This report describes the first diagnosis, by pathology, PCR and sequencing approaches, of CpHV-2 infection in a severe MCF incidence in Formosan sika deer（*Cervus nippon taiouanus*）in Taiwan.

Fourteen 6-month-old Formosan sika deer were purchased from a captive deer farm in southern Taiwan in early July 2002. The deer were then kept with 16 cattle and 30 pregnant domestic goats that were either near term or kidding. These animals were housed in separate pens in one barn without direct contact and were tended by an attendant. Twelve of the 14 sika deer died between September 2002 and January 2003. Clinical signs were anorexia, depression, seizures, weight loses, mild ocular discharge, conjunctivitis, and corneal opacity. Death occurred within 1 month of the onset of clinical signs. Two deer remained clinically normal until December 2003. Goats and cattle that shared housing with the Formosan sika deer also remained clinically normal.

At necropsy, the principal gross lesion was markedly increased thickness of the arterial wall. The coronary arteries（Fig. 1）and those in the mesentery and kidneys were affected the most. Other lesions included petechial to paint brush hemorrhages along the coronary groove and in the left ventricle wall of the heart and in the serosa of small intestine and colon. Multiple necrotic foci were seen in the liver. White spots on renal cortex and conjunctival hyperemia were also observed. In addition, there were mucosal erosions in oral and nasal cavities and in rumen, omentum, and reticulum.

Microscopic lesions in the dead deer were severe, extensive, and consistent. There was marked lymphoplasmacytic meningoencephalitis in the cerebrum and cerebellum. Lymphocytic vasculitis to perivasculitis（Fig. 2）with fibrinoid mural necrosis and intimal hyperplasia was observed in the heart（Fig. 3）, brain, kidneys, mesenteric lymph node, aboma-

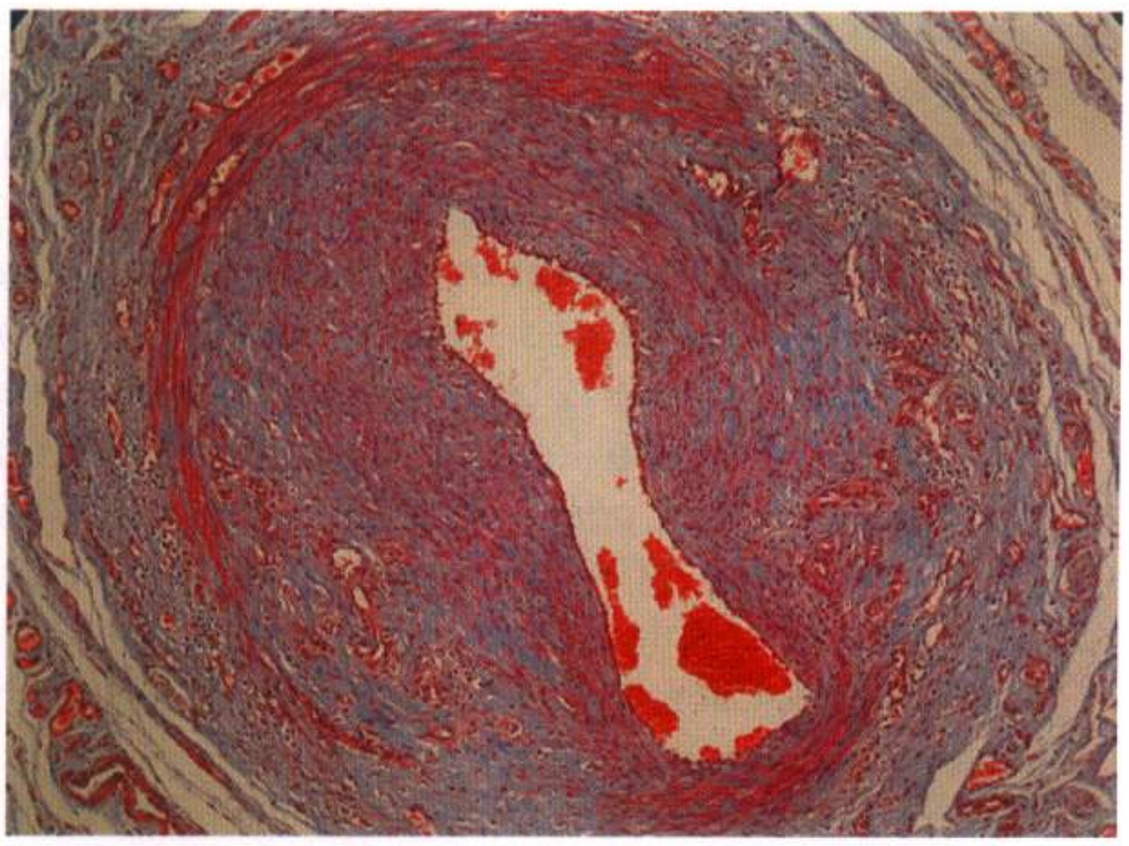

Fig. 2 Microscopic lesions of Fig. 1. with severe intimal hyperplasia of coronary artery（Trichrome stain）. X200.

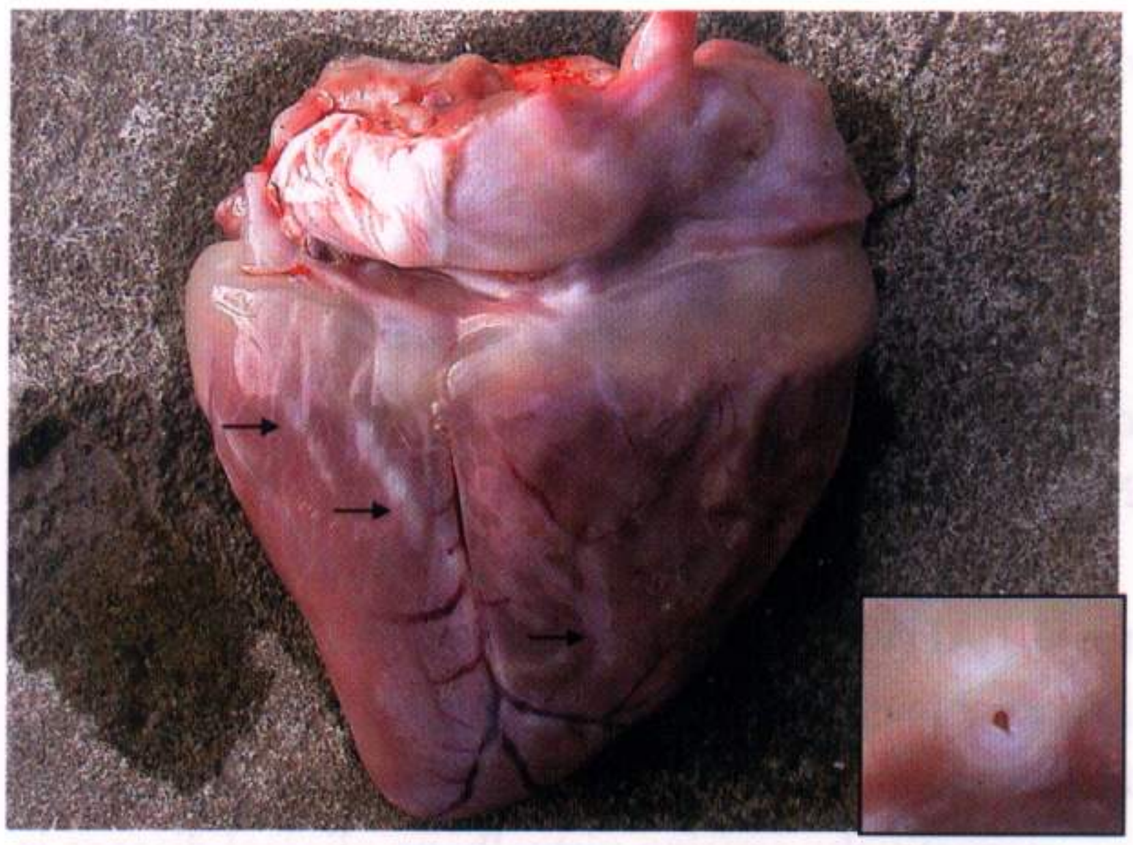

Fig. 1 Markedly thickened coronary arteries as indicated by arrows（inset: the cross section of a coronary artery）.

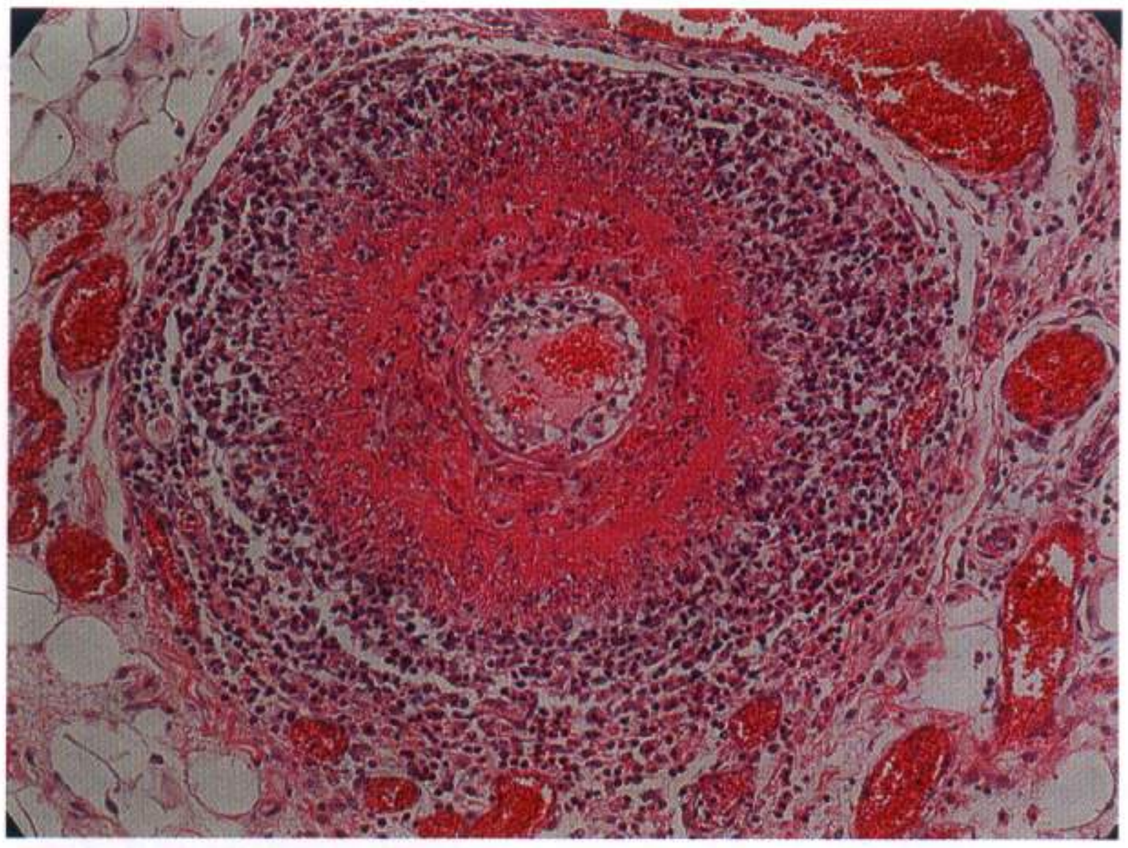

Fig. 3 Severe arteritis with fibrinoid necrosis and marked perivascular lymphoplasmacytic cuffing in the mesentery（H&E）. X200

sum, and ileum. Foci of mineralization, associated with hemorrhages, were presented in the walls of some necrotic vessels and myocardium.

Serology for antibodies to epizootic hemorrhagic disease and bluetongue viruses were negative, but were positive for MCF virus (VMRD, Inc.) in all deer and goats housing in the same barn.

Samples of DNA extracted from the peripheral blood lymphocytes (PBL) of 7 deer, spleen and lymph nodes of all 12 affected deer, as well as samples of DNA from PBL of goats, cattle and the 2 alive sika deer were subjected to PCR amplification. The procedures for DNA extraction using commercial kits were as described previously [7]. The purified DNA samples were stored at -20℃ until tested.

The PCR was performed to identify the presence of MCF virus using the consensus primers [10] of a conserved region of the herpesviral DNA polymerase gene and the primers [7] specific for CpHV-2. The consensus PCR produced a product of approximately 230 base pairs (bp) (Fig. 4) from PBL and tissues of all 14 deer and all commingled goats. The PCR products from all positive animals were sequenced and exhibited 100% identity. When the sequences were compared with that of the polymerase gene of CpHV-2 from GeneBank (AF275941), they also matched 100% (data not shown). When primers specific for CpHV-2 were used, a product of approximately 108 bp (Fig 5) was detected from the PBL or tissues of all 14 deer and all commingled goats. However, samples from the cattle showed negative for these two PCR assays (data not shown).

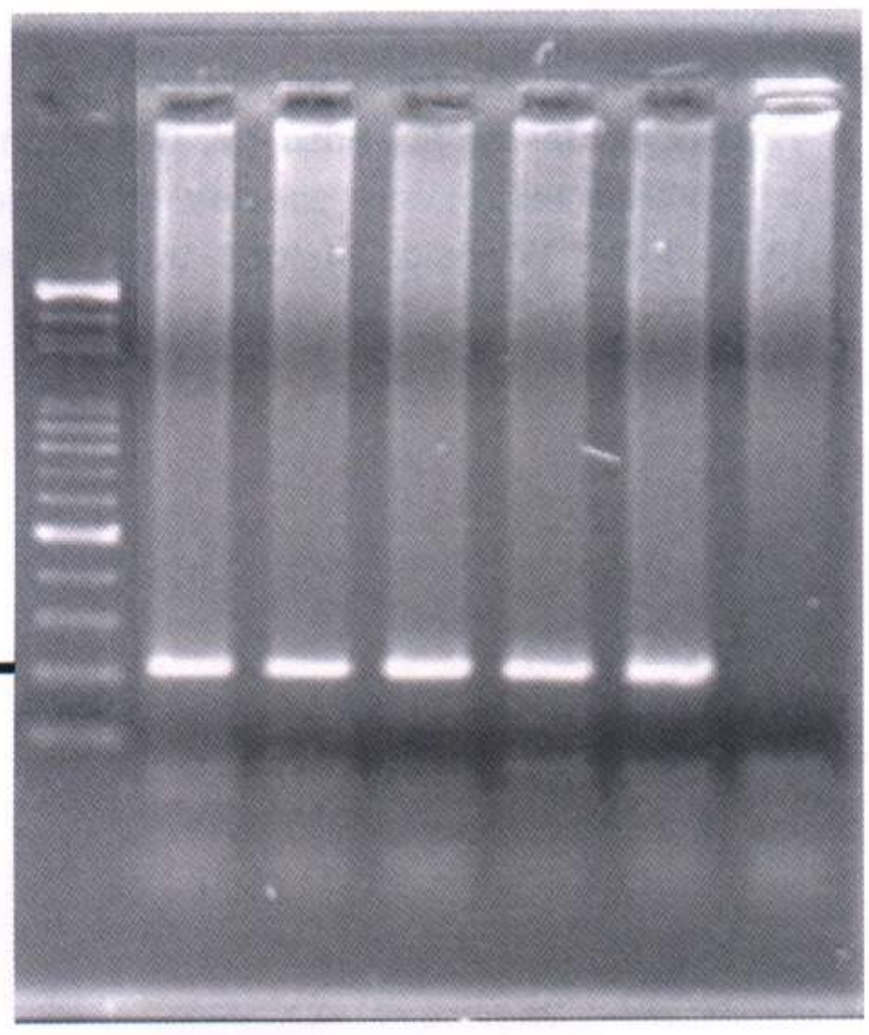

Fig. 4 Herpesvirus consensus PCR products analyzed by agarose gel electrophoresis. Lane M, 100-bp DNA ladder; lanes 1-4, blood samples from dead Formosan sika deer; lane 5, positive control from sequenced MCF PCR product; lane 6, negative control.

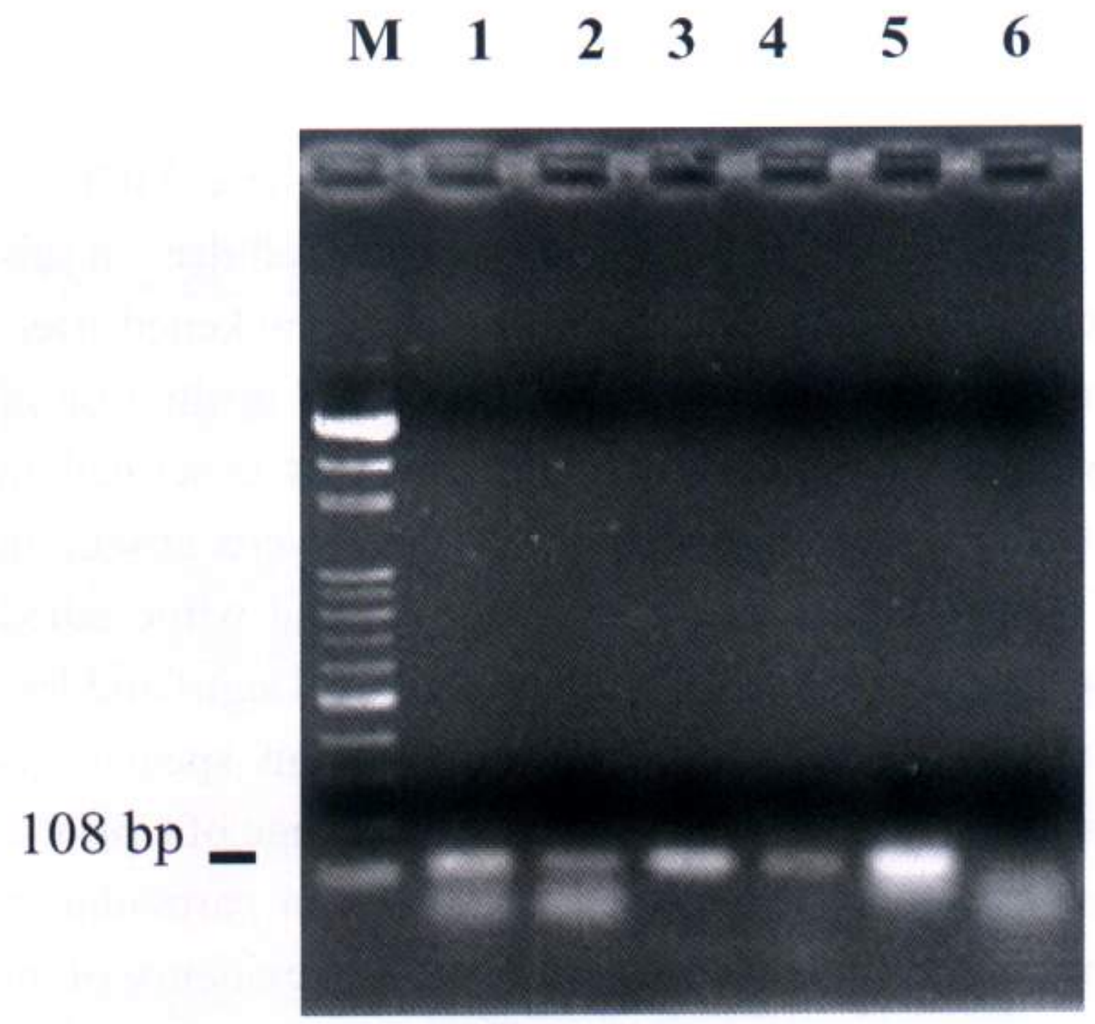

Fig. 5 Agarose gel electrophoresis of ethidium bromide-stained CpHV-2-specific PCR products. Lane M, 100-bp DNA ladder; lanes 1-4, blood samples from dead sika deer; lane 5, positive control from sequenced MCF PCR product; lane 6, negative control.

This is the first report of goat-associated MCF in Taiwan. The clinical findings in the present study are similar to those of chronic goat-associated MCF in sika deer [7] and white-tailed deer [8] which had weight loss and emaciation. This report confirms that goat-associated MCF can cause chronic infection in deer.

A distinct feature in the present incidence different from the previous findings [4, 8] was that 2 of 14 deer with typical MCF, and 10 of 14 that survived for extended periods within 30 days. Meanwhile, 2 of 14 didn't showed any clinical signs. In this incidence, serology indicated that all deer and goats in the same barn were infected by MCF. The mortality was also as high as that described in sheep-associated MCF in white-tailed deer [2] with some deer recovered. There is no explanation for the different clinical outcomes among various animals with MCF in this incidence, although there were reports of MCF in deer that recovered [5].

Corneal edema, described in sheep-associated MCF in cattle and cervid species [9], has not been described in previous cases of goat-associated MCF [7, 8], but was observed in 2 of the diseased deer in this study. The current cases had markedly thickened arterial wall, due to intimal hyperplasia and infiltration of lymphocytes and some neutrophils, as described in axis deer [3]. The vascular lesions were absent in CpHV-2 infections of sika deer [7] and white-tailed deer [8] indicating that different clinical signs and lesions may be observed among different species affected by MCF. Meanwhile, the host range of CpHV-2 remains to be determined. However, in particular in the present incidence, cattle showed no evidence of infection as indicated by the negative C-ELISA (VMRD, Inc.) and PCR results; even when they were housed with goats and deer. Therefore, unlike that found in sheep-associated MCF virus which caused infection in both deer and cattle [6], cattle are not susceptible to CpHV-2 associated MCF by indirect contact.

This report shows that serious loss can occur in the outbreak of CpHV-2-associated MCF in deer and it also provides further evidence that goats are the source of infection. Since no vaccine is available for MCF, deer producers should try to avoid contacts between deer and goats to prevent the infection of CpHV-2.

REFERENCES

1. Audige L, Wilson PR, Morris RS. Disease and mortality on red deer farms in New Zealand. Vet Rec 148: 334-340, 2001.
2. Brown CC and Bloss LL. An epizootic of malignant catarrhal fever in a large captive herd of white-tailed deer (*Odocoileus virginianus*). J Wildl Dis 28:301-305, 1992.
3. Clark KA and Robinson RM. Malignant cararrhal fever in Texas cervids. J Wildl Dis 6:336-383, 1970.
4. Crawford TB, Li H, Rosenburg SR, Norhausen RW, Garner MM. Mural folliculitis and alopecia caused by infection with goat-associated malignant catarrhal fever virus in two sika deer. J Am Vet Med Assoc 22:843-847, 2002.
5. Heuschele WP, Nielsen NO, Oosterhuis JE, Castro AE. Dexamethasone-induced recrudescence of malignant catarrhal fever and associated lymphosarcoma and granulomatous disease in a Formosan sika deer (*Cervus nippon taiouanus*). Am J Vet Res 46:1578-1583, 1985.
6. Imai K, Nishimori T, Horino R, Kawashima K, Murata H, Tsunemitsu H, Saito T, Katsuragi K, Yaegashi G. Experimental transmission of sheep-associated malignant catarrhal fever from sheep to Japanese deer (*Cervus nippon*) and cattle. Vet Microbiol 79:83-90, 2001.
7. Keel MK, Patterson JG, Noon TH, Bradley GA, Collins JK. Caprine herpesvirus-2 in association with naturally occurring malignant catarrhal fever in captive sika deer (*Cervus nippon*). J Vet Diagn Invest 15:179-183, 2003.
8. Li H, Wunschmann A, Keller J, Hall G, Crawford TB. Caprine herpesvirus-2-associated malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). J Vet Diagn Invest 15:46-49, 2003.
9. Radostits OM, Gay CC, Blood DC, Hinchcliff KW. Malignant catarrhal fever. In: Veterinary Medicine. 9th ed. Harcourt Publishers Ltd. pp. 1081-1085, 2000.
10. VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM. Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol 34:1666-1671, 1996.

簡訊：台灣圈飼梅花鹿感染山羊第二型疱疹病毒惡性卡他熱

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摘要　本篇報告為首次以聚合酶鏈反應及基因定序診斷台灣本地梅花鹿因感染山羊第二型疱疹病毒所引發之慢性惡性卡他熱臨床症狀與病理特徵。在 2002 年九月到 2003 年元月間，圈養在一起的鹿群中有 12 頭死於逐漸消瘦，另外兩頭無可見病狀。剖檢肉眼病變有心、腸繫膜淋巴結與腎的明顯動脈硬化；12 頭動物都有嚴重淋巴球性血管炎，伴隨血管內膜增生、與慢性惡性卡他熱吻合的顯微病理病變。感染鹿與圈飼於同舍不同欄的山羊血清中，均可檢出惡性卡他熱抗體，這些外表健康的山羊於山羊第二型疱疹病毒的聚合酶鏈反應檢測均呈陽性，但圈飼於同舍不同欄的牛隻檢測結果則呈陰性。推測本案例中無臨床症狀、但為山羊第二型疱疹病毒帶原者的健康山羊是造成鹿隻感染發病的病毒來源。[陳世平、李明昌、孫豫芬、陳啟銘、楊平政、*鄭益謙。台灣圈飼梅花鹿感染山羊第二型疱疹病毒惡性卡他熱。台灣獸醫誌 33 (3&4): 162-166，2007。*聯絡人 TEL: (037)585-868，FAX: (037)585-850，E-mail: icc01@mail.atit.org.tw]

鍵詞：惡性卡他熱，山羊第二型疱疹病毒，聚合酶鏈反應