

# Using of Immunohistochemistry Technique for Detection of Foot and Mouth Disease Virus (FMDV) Type O in Cattle Tissues

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## Abstract

Detection of FMDV type O antigen using Immunohistochemistry (IHC) technique was performed on formalin-fixed, paraffin-embedded (FFPE) tissues obtained from cattle originally intradermolingual inoculated with FMDV type O strain O Udonrthani/87, FMDV type A strain A Lopburi/2012 and FMDV type Asia-1 strain Asia-1 Nakornpathom/85 to consider the cross reaction. The number of animals used for these inoculations were 5, 2 and 2 healthy 6 month old cattle respectively. The primary antibody used in this study was mouse against FMDV type O monoclonal antibody. The positive IHC results were only found in cattle tissues inoculated with FMDV type O strain O Udonrthani/87 which moderate to strong positive results were observed in the epithelial cells of sloughing and necrotic tongue epithelium (5/5) and stratum spinosum and stratum basale of the interdigital skin (2/5), in addition, the mild positive results were noticed in a few epithelial cells of the dorsal soft palate (1/5). The important microscopic changes in the organs and tissues of cattle inoculated with FMDV type O strain O Udonrthani/87 were necrosis with sloughing and sub-acute to chronic inflammation of tongue (5/5), interdigital skin (3/5), gum (2/5) and larynx (2/5) and chronic inflammation of epiglottis (1/5) and dorsal soft palate (1/5). From the current study, IHC technique showed magnificent results to detect the antigen of FMDV type O without cross reaction, in addition, the IHC positive reaction findings showed the ability of mouse against FMDV type O monoclonal antibody to particularly detect the antigen of the virus even small amounts in FFPE tissues. Furthermore, tongue and interdigital skin with sloughing and necrosis were significant tissues to detect more FMDV antigen. In conclusion, the IHC technique is an alternatively diagnostic method for detection of FMDV antigen since it is specific, sensitive and able to be performed completely within 2 days after obtaining FFPE tissue blocks.

Key words : FMDV type O antigen, cattle tissues, IHC technique, tongue, interdigital skin

## Introduction

Foot and mouth disease (FMD), caused by FMD virus (FMDV), is the most contagious disease and has a great potential for causing severe economic loss of all cloven hoofed animals of the order Artiodactyla include cattle, swine, sheep, goats, water buffaloes and yak, as well as in wildlife or captive wildlife of this order including African buffalo, bison, moose, elephants, chamois, giraffes, wildebeest, blackbuck, warthogs, kudu, impala and several species of deer, antelopes and gazelles. Experimental infections could induce in alpacas, llamas and Bactrian camels. Besides, FMDV is able to infect hedgehogs, bears, armadillos, kangaroos, nutrias and capybaras that are the animals out of the Artiodactyla (CFSPH, 2015). The disease is endemic in many countries in Asia, Africa, Middle East, some part of Europe and some countries in South America, except in Australia, New Zealand, Indonesia, Middle America and North America. Vesicular formation in and around the mouth and buccal mucosa, on the feet, teats and mammary glands is the hallmark lesions and cause severe illness in animals especially when the vesicles rupture. The disease is notable for high morbidity and very low mortality except in sucklings which are easily dead because of myocarditis (seen as tiger-heart) and lacking of milk from their mothers. In addition, the animals are suffered and their production of meat and milk are declined for long period (Arzt et al., 2011; OIE, 2012; OIE, 2015; The Merck Veterinary Manual, 2015).

FMDV is ss RNA virus of the genus *Aphthovirus* in the Picornaviridae family. The virion is an icosahedral particle and measures approximately 22 nm. It is highly tolerant to various circumstances but is sensitive or inactivated by sunlight and moderate acidity. There are 7 serotypes of FMDV, namely O, A, C, SAT-1, SAT-2, SAT-3, and Asia-1 which the occurrent areas are different such as O, A, C, SAT-1, SAT-2 and SAT-3 occur in Africa; O, A, C and Asia-1 in Asia; and O, A, C in Europe and South America. The infection with any one serotype does not confer immunity against another (Brown et al., 2007; Callis and Gregg, 1992; Mann and Sellers, 1990). The virus spreads from sick animals through direct contact and respiration, importantly, FMDV is easily distribution by various situations such as the importation of infected animals, the contamination of FMDV on the barns, vehicles, materials in the farms and clothes, as well as wind. (OIE, 2015).

The significant route of transmission is inhalation of aerosolized virus. The virus replicates in pharynx, soft palate (Monaghan et al., 2005) and nasopharyngeal tissues (Windsor et al., 2011) then spread via blood to the surface epithelium and develop the hallmark lesions.

The existent of the virus in the lesions is seldom beyond day 11 of clinical signs. (Brown et al., 2007). There are various FMDV or FMD viral RNA detected tissues which are able to use for diagnosis of FMD such as dorsal nasopharyngeal, dorsal soft palate, palatine tonsil, epiglottis, larynx, oesophageal-pharyngeal tissue, mandibular lymph node, retropharyngeal lymph node, thyroid, trachea, lung, cardiac muscle, tongue epithelium and interdigital skin of dead animals (Arzt et al., 2010; Arzt et al., 2011; Arzt et al., 2014; Juleff et al., 2008; OIE, 2012; Phacheco et al., 2015; Tunca et al., 2008). Cattle are carrier since FMDV is able to survive in oesophageal-pharyngeal part of animals more than 28 days or until 3 years after, meanwhile, goats and sheep are carrier for a few months but swine is not (OIE, 2012).

There are several diagnostic techniques for FMD such as virus isolation, serology using enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT) and nucleic acid recognition methods using RT-PCR or Real time RT-PCR (IAEA, 2007; Longjam et al., 2011; OIE, 2012). However, these techniques are inadequate for providing information regarding microscopic viral localization to specific anatomic tissue regions or individual cell types. When such data are required, investigators may utilize immunohistochemistry (IHC) technique, fluorescent antibody technique (FAT), in situ hybridization (ISH) or FA-confocal microscopy (CFM).

In Thailand, 3 types of FMDV include O, A and Asia-1 are sporadic in some part of the country. The diagnosis is performed by conventional methods compose of virus isolation, ELISA, CFT, RT-PCR or Real time RT-PCR, however, IHC technique has not been established. IHC technique is very useful for pathological study since it can detect antigens in tissue sections by means of immunological and chemical reactions. In addition, it is highly sensitive and specific and can detect a wide variety of antigens in multiple animal species (Ramos-Vara, 2011). In this study, we stained formalin-fixed, paraffin-embedded (FFPE) tissues of bovine origin inoculated with FMDV type O by IHC technique using mouse anti-FMDV type O monoclonal antibody to detect FMDV type O antigen and confirm specificity by checking cross reaction in different FMDV-FFPE tissues of bovine origin inoculated with FMDV type A and Asia-1.

## Materials and Methods

43

### Source of samples

All work described herein was performed within the biosafety level 3 containment facility at the Bureau of Veterinary Biologics, Department of Livestock Development, Thailand. Ten young Thai-native healthy cattle aged 6 months were used in this study; cattle no. 1-5, 6-7 and 8-9 were inoculated via intradermolingual route with FMDV type O strain O Udonrthani/87, FMDV type A strain A Lopburi/2012 and FMDV type Asia-1 strain Asia-1 Nakornpathom/85 amount  $10^4$  BID<sub>50</sub> (50% bovine infectious dose) respectively, in addition, cattle no. 10 was inoculated with phosphate buffered saline used as a control animal. The cattle no. 1-5 were studied on gross findings, histopathological examination and IHC technique. Meanwhile, the cattle no 6-10 were mainly used as to consider the cross reaction of the IHC technique, therefore, they were studied only on gross findings and IHC technique. After FMDV inoculation, all cattle were observed clinical signs and lesions for 5 days and were euthanized by over dosage of sodium pentobarbitone. Necropsy and routine histopathology were performed. Tissue specimens from dorsal nasopharyngeal, dorsal soft palate, palatine tonsil, epiglottis, larynx, oesophageal-pharyngeal tissue, mandibular lymph node, mediastinal lymph node, trachea, tongue, interdigital skin, lung, cardiac muscle, liver, spleen, kidney and gum were collected, fixed in 10% neutral buffered formalin and sent to pathology laboratory, National Institute of Animal Health. Meanwhile, the tissue specimens from all cattle such as tongue and interdigital skin were sent to Regional Reference Laboratory for Foot and Mouth Disease in South East Asia (RRL) to confirm FMDV by ELISA typing.

### Sample preparation for ELISA typing and ELISA typing

The sample preparation for ELISA typing and ELISA typing performed as the methods of Roeder and LeBlanc Smith (1987).

### Pathological samples

FFPE tissue blocks from the collected specimens were done and were serial sectioned for 3 tissue sections at 2-6 micron thickness, then one tissue section was mounted on a glass slide for haematoxylin & eosin (H&E) stain (Luna, 1968) and the remaining were mounted on 2 electro-statically charged glass slides for IHC stain. All tissue sections were incubated in oven at 60°C for 30 minutes and stored at room temperature.

### Primary antibody used for IHC technique

Mouse against FMDV type O monoclonal antibody was used as a primary antibody to detect FMDV type O antigen in FFPE tissue sections. The antibody was contributed by National

Institute of Animal Health (NIAH), National Agriculture and Food Research Organization (NARO), Japan.

#### **FFPE tissue control block used for IHC technique**

FFPE-FMDV type O tissue control block kindly contributed by NIAH, NARO, Japan was used as control block. They were serial sectioned and mounted on 2 electro-statically charged glass slides for IHC stain running with all collected tissue section specimens.

#### **IHC technique**

The IHC technique was applied from Batson (2008). In the details, all FFPE tissue-sections were deparaffinized in 3 changes of xylene 3 minutes each, then rehydrated by transferring into 100% ethanol for 3 changes 3 minutes each followed by 95% ethanol for 3 changes 3 minutes each and put into distilled water for 5 minutes. The sections were dipped into 10mM sodium citrate buffer and performed antigen retrieval by microwave oven at 500 watts 5 minutes twice, then cooled down for 20 minutes and brought to running water for 3 minutes. To block endogenous peroxidase activity, the sections were incubated in 3% hydrogen peroxide in methanol solution at room temperature for 30 minutes. After that, they were rinsed in 150 ml of phosphate buffered saline (PBS) for 3 changes 5 minutes each. To perform protein blocking, 10% normal goat serum followed by skim milk 20 minutes each were used. Then, the sections were incubated overnight in moisture chamber with mouse against FMDV type O monoclonal antibody (NIAH, NARO, Japan) dilution 1:8 at 4°C in refrigerator. Meanwhile, duplicates or negative control tissue sections were stained identical treatment with the exception of primary antibody. After another rinsing in PBS for 3 changes 5 minutes each, horseradish peroxidase (HRP) - labeled dextran polymer conjugated to immunoglobulins (Polymer/HRP) (EnVision™ system, rabbit/mouse, DAKO, Denmark, A/S) was dropped onto the slides in moisture chamber for 15 minutes. The sections were again rinsed in 150 ml of PBS for 3 changes 5 minutes each, followed by shortly rinsed in distilled water. Then, the sections were added 3-amino-9-ethylcarbazole (AEC) as substrate-chromogen in moisture chambers for 5 minutes and shortly rinsed in distilled water twice. The sections were counterstained with Mayer's haematoxylin for 5-10 seconds, brought to running water for 10 minutes, put into distilled water, mounted with glycergel (DakoCytomation.; Carpinteria, CA, USA) and investigated under light microscope. Determination of a positive IHC result required the presence of a cell-associated signal with no similar signal present on the negative controls from the same run.

## Results

### Clinical and gross findings

All cattle except cattle no. 10 showed clinical signs 1-2 days after inoculation include pyrexia of ~104° F. Then, FMD vesicles revealed in tongue epithelium, interdigital skin and gum within 2 – 3 days after inoculation followed by rapidly ruptured and created ulcerative or erosion and necrotic lesions in some parts of the body. In the details, slightly to severe erosion and necrosis revealed in tongue, interdigital skin and gum of cattle no. 1-8; 1, 2, 3, 6 and 9; and 3-4 respectively (Table 1).

### FMDV ELISA typing

FMDV ELISA typing of cattle no. 1–5, 6-7, 8-9 and 10 revealed FMDV type O, A, Asia-1 and negative respectively (Table 2).

### Microscopic findings in FMDV type O strain O Udonthani/87 inoculated group

Microscopic changes (as shown in table 3) revealed in tongue epithelium (5/5), interdigital skin (3/5), gum (2/5), larynx (2/5), dorsal soft palate (1/5), epiglottis (1/5), trachea (3/5), lung (5/5), kidney (4/5), spleen (2/5), mediastinal lymph node (4/5), and mandibular lymph node (4/5), meanwhile, the remaining tissues include dorsal nasopharyngeal, coronary band, cardiac muscle, palatine tonsil, oesophageal-pharyngeal tissue and liver showed no specific lesions. The details of the microscopic findings characterized by severely necrosis with sloughing in epithelium and sub-acute to chronic inflammation in sub-epithelium of tongue (Figure 1 A, C, E, G and I), severely necrosis with sloughing in epidermis and sub-acute to chronic inflammation in dermis of interdigital skin (Figure 2 A, C, E, G, I and K), severely necrosis with sloughing in epithelium and sub-acute to chronic inflammation in sub-epithelium of gum, slightly necrosis with sloughing in epithelium and sub-acute to chronic inflammation in lamina propria of larynx, mild chronic inflammation in sub-epithelium of dorsal soft palate (Figure 3 A) and slightly chronic inflammation of epiglottis. Trachea revealed slightly sub-acute to chronic tracheitis. Lungs and kidneys revealed moderately sub-acute interstitial pneumonia and slightly to moderately chronic interstitial nephritis respectively. Spleen was found slightly lymphoid depletion in white pulp. Mediastinal and mandibular lymph nodes showed slightly lymphoid depletion and necrosis in lymphatic follicles.

### IHC analysis in FMDV inoculated cattle

The antigen of FMDV type O was detected in FFPE-FMDV type O tissue control sections and FFPE tissue sections from FMDV type O strain O Udonthani/87 inoculated group (cattle

no. 1-5) without background, meanwhile, no detectable signals were found in FFPE tissue sections from FMDV type A strain A Lopburi/2012 inoculated group (cattle no. 6-7), FMDV type Asia-1 strain Asia-1 Nakornpathom/85 inoculated group (cattle no. 8-9) and cattle no. 10 (Table 4). The positive IHC result revealed the presence of a cell-associated signal in cytoplasm of various tissues composed of tongue, interdigital skin and dorsal soft palate of cattle no. 1-5, no. 1 and 3, and no. 5 respectively. In the tongues there were strong staining in the epithelial cells (Figure 1 B, D, F, H and J), as well as, in the stratum spinosum and stratum basale of the interdigital skin (Figure 2 B, D, F, H, J and L). In the dorsal soft palate, there were mild staining in a few epithelial cells (Figure 3 B, D and F).

## Discussion

In this study, the IHC staining results of all tissue sections were found that the mouse against FMDV type O monoclonal antibody (NIAH, NARO, Japan) provided excellent sensitivity and specificity without background in FFPE tissue sections from FMDV type O strain O Udonrthani/87 inoculated group without cross reaction in FFPE tissue sections from FMDV type A strain A Lopburi/2012 and FMDV type Asia-1 strain Asia-1 Nakornpathom/85 inoculated group. In FMDV type O strain O Udonrthani/87 inoculated group, the most strong positive IHC results revealed the presence of a cell-associated signal in cytoplasm of epithelial cells in necrotic area of tongue, as well as, in the particular layers of epidermis of the interdigital skin include stratum spinosum and stratum basale, meanwhile, there were mild staining in a few epithelial cells of the dorsal soft palate which was similar to Arzt et al. (2009). The remaining organs in buccal cavity revealed IHC negative staining particularly in pharyngeal and nasopharyngeal tissues which is the initial replication sites of FMDV (Alexandersen et al., 2003; Windsor et al., 2011) as well as the earliest site of microscopic localization of FMDV (Arzt et al., 2010). Since the virus was demonstrated in the pharyngeal tissues for 1-3 days before viremia, after that the virus entered through regional lymph nodes and circulation except reinfection of pharyngeal tissues which the virus would exist longer (Alexandersen et al., 2003). Histopathological lesions of trachea, lung, kidney, spleen, mediastinal and mandibular lymph nodes composed of chronic tracheitis, sub-acute interstitial pneumonia, chronic interstitial nephritis and lymphoid depletion respectively, however, these lesions were not mentioned in animals infected with FMD and not specific to indicate the diseases. IHC negative staining were presented in these organs. The result was similar to the study of Alexandersen et al.

(2003) which mentioned that no more than negligible amounts of virus was produced in these organs, but different from Arzt et al. (2010) which found IHC positive in alveolar lumina or adhered to septal wall of lung. For the significant lesion, myocarditis (seen as tiger-heart), could not been found in all cattle because they were not suckling animal (Kitching, 2002). From these results revealed that the target cells of FMDV type O inoculation by intradermolingual route were epithelial cells of tongue and epidermis of the interdigital skin which FMDV replicated to a high level and lesions normally formed which is as same as Monaghan et al. (2005). For the IHC technique, there are various methods for the antigen retrieval step such as using enzymes, microwave and autoclave, our study used microwave which was done well. In addition, we used 3-amino-9-ethylcarbazole (AEC) as chromogen to present the positive reaction, resulting that the red colour of AEC made obviously observation and well differentiated from black color of melanin pigments which is easily found in animal skin.

According to OIE (2012) laboratory diagnosis is usually performed via antigen capture-ELISA or serotyping ELISA which is the preferred method for countries with endemic FMD for viral antigen detection and serotyping, however, IHC technique is useful for the detection of FMDV in infected tissues (Arzt et al., 2009; Gulbahar et al., 2007; Moniwa et al., 2012; Stenfeldt et al., 2014) because its positive reactions indicate the viral antigens in infected cells. From the current study, IHC technique presented magnificent results which we could obviously seen the viral antigen in the infected cells even in the few antigen aggregated epithelial cells of dorsal soft palate. Importantly, the mouse against FMDV type O monoclonal antibody was the key element which was able to diagnose and confirm FMDV type O in suspected tissues. The tissues of choice should be tongue and interdigital skin particularly revealed erosive and necrotic lesions. This technique is able to use for alternative diagnosis since it is specific and sensitive and it can shortly been performed within 2 days started from FFPE tissue blocks.

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Table 1. Gross findings of FMDV inoculated cattle.

|                   | Cattle                                             |      |    |    |    |                                                    |     |                                                                 |    |         |
|-------------------|----------------------------------------------------|------|----|----|----|----------------------------------------------------|-----|-----------------------------------------------------------------|----|---------|
|                   | FMDV type O strain O Udonthani/87 inoculated group |      |    |    |    | FMDV type A strain A Lopburi/2012 inoculated group |     | FMDV type Asia-1 strain Asia-1 Nakornpathom/85 inoculated group |    | Control |
| Tissues           | 1                                                  | 2    | 3  | 4  | 5  | 6                                                  | 7   | 8                                                               | 9  | 10      |
| Tongue            | ++++                                               | ++++ | +  | ++ | ++ | ++++                                               | +++ | ++                                                              | -  | -       |
| Interdigital skin | ++++                                               | ++   | +  | -  | -  | ++                                                 | -   | -                                                               | ++ | -       |
| Gum               | -                                                  | -    | ++ | ++ | -  | -                                                  | -   | -                                                               | -  | -       |

A determination of the severity of erosion and necrosis in each tissue was performed: - = no lesions; + (slightly) to ++++ (severe).

Table 2. FMDV ELISA typing results of FMDV inoculated cattle.

|                      | Cattle                                             |   |   |   |   |                                                    |   |                                                                 |        |          |
|----------------------|----------------------------------------------------|---|---|---|---|----------------------------------------------------|---|-----------------------------------------------------------------|--------|----------|
|                      | FMDV type O strain O Udonthani/87 inoculated group |   |   |   |   | FMDV type A strain A Lopburi/2012 inoculated group |   | FMDV type Asia-1 strain Asia-1 Nakornpathom/85 inoculated group |        | Control  |
|                      | 1                                                  | 2 | 3 | 4 | 5 | 6                                                  | 7 | 8                                                               | 9      | 10       |
| ELISA typing results | O                                                  | O | O | O | O | A                                                  | A | Asia-1                                                          | Asia-1 | Negative |

Table 3. Microscopic findings from FMDV type O strain O Udonrthani/87 inoculated group

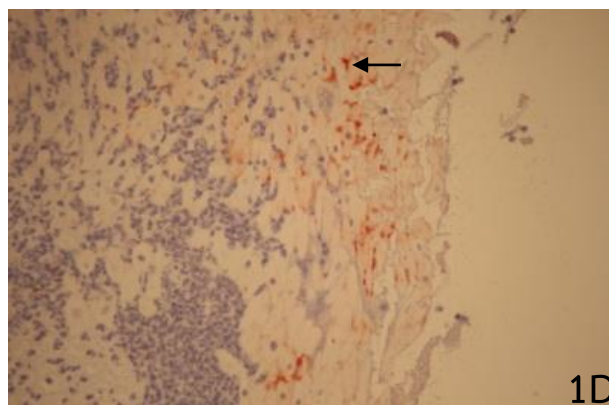
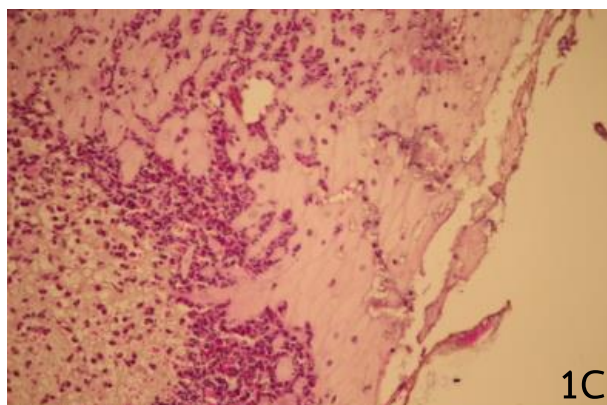
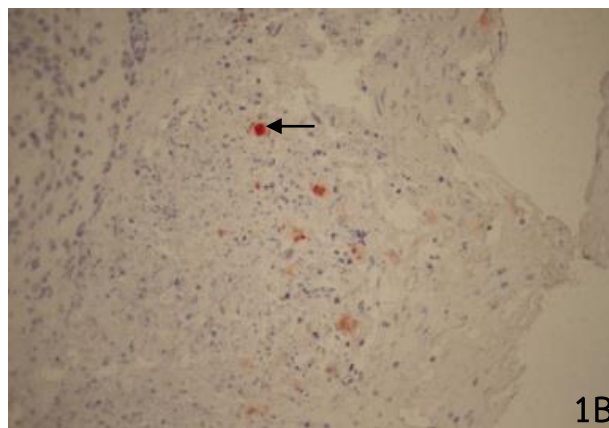
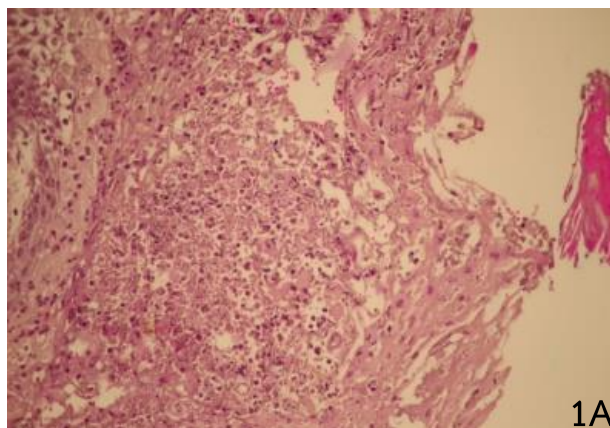
| Tissues                | Histopathological findings                                                                    | FMDV type O strain O Udonrthani/87 inoculated group |      |      |      |      |
|------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------------|------|------|------|------|
|                        |                                                                                               | 1                                                   | 2    | 3    | 4    | 5    |
| Tongue                 | Necrosis with sloughing in epithelium and sub-acute to chronic inflammation in sub-epithelium | ++++                                                | ++++ | ++++ | ++++ | ++++ |
| Interdigital skin      | Necrosis with sloughing in epidermis and sub-acute to chronic inflammation in dermis          | ++++                                                | ++++ | ++++ | -    | -    |
| Gum                    | Necrosis with sloughing in epithelium and sub-acute to chronic inflammation in sub-epithelium | -                                                   | -    | ++++ | ++++ | -    |
| Larynx                 | Necrosis with sloughing in epithelium and sub-acute to chronic inflammation in lamina propria | -                                                   | +    | +    | -    | -    |
| Dorsal soft palate     | Chronic inflammation in sub-epithelium                                                        | -                                                   | -    | -    | -    | +    |
| Epiglottis             | Chronic inflammation                                                                          | -                                                   | +    | -    | -    | -    |
| Trachea                | Sub-acute to chronic tracheitis                                                               | +                                                   | +    | -    | +    | -    |
| Lung                   | Sub-acute interstitial pneumonia                                                              | ++                                                  | ++   | ++   | ++   | ++   |
| Kidney                 | Chronic interstitial nephritis                                                                | +                                                   | -    | +    | +    | ++   |
| Spleen                 | Lymphoid depletion in white pulp                                                              | -                                                   | -    | +    | -    | +    |
| Mediastinal lymph node | Lymphoid depletion and necrosis in lymphoid follicles                                         | +                                                   | -    | +    | +    | +    |
| Mandibular lymph node  | Lymphoid depletion and necrosis in lymphoid follicles                                         | +                                                   | +    | +    | -    | +    |

A determination of the severity of microscopic findings in each tissue was performed: - = no lesions; + (slightly) to ++++ (severe).

Table 4. Immunohistochemical analysis for foot and mouth disease virus (FMDV) in FMDV inoculated cattle.

| Tissues            | Cattle                                              |   |     |   |    |                                                    |   |                                                                 |   |         |
|--------------------|-----------------------------------------------------|---|-----|---|----|----------------------------------------------------|---|-----------------------------------------------------------------|---|---------|
|                    | FMDV type O strain O Udonrthani/87 inoculated group |   |     |   |    | FMDV type A strain A Lopburi/2012 inoculated group |   | FMDV type Asia-1 strain Asia-1 Nakornpathom/85 inoculated group |   | Control |
|                    | 1                                                   | 2 | 3   | 4 | 5  | 6                                                  | 7 | 8                                                               | 9 | 10      |
| Tongue             | +                                                   | + | +   | + | ++ | -                                                  | - | -                                                               | - | -       |
| Interdigital skin  | ++++                                                | - | +++ | - | -  | -                                                  | - | -                                                               | - | -       |
| Dorsal soft palate | -                                                   | - | -   | - | +  | -                                                  | - | -                                                               | - | -       |

A determination of the intensity of positive reaction findings in each tissue was performed: - = negative; + (slightly) to ++++ (severe) positive.





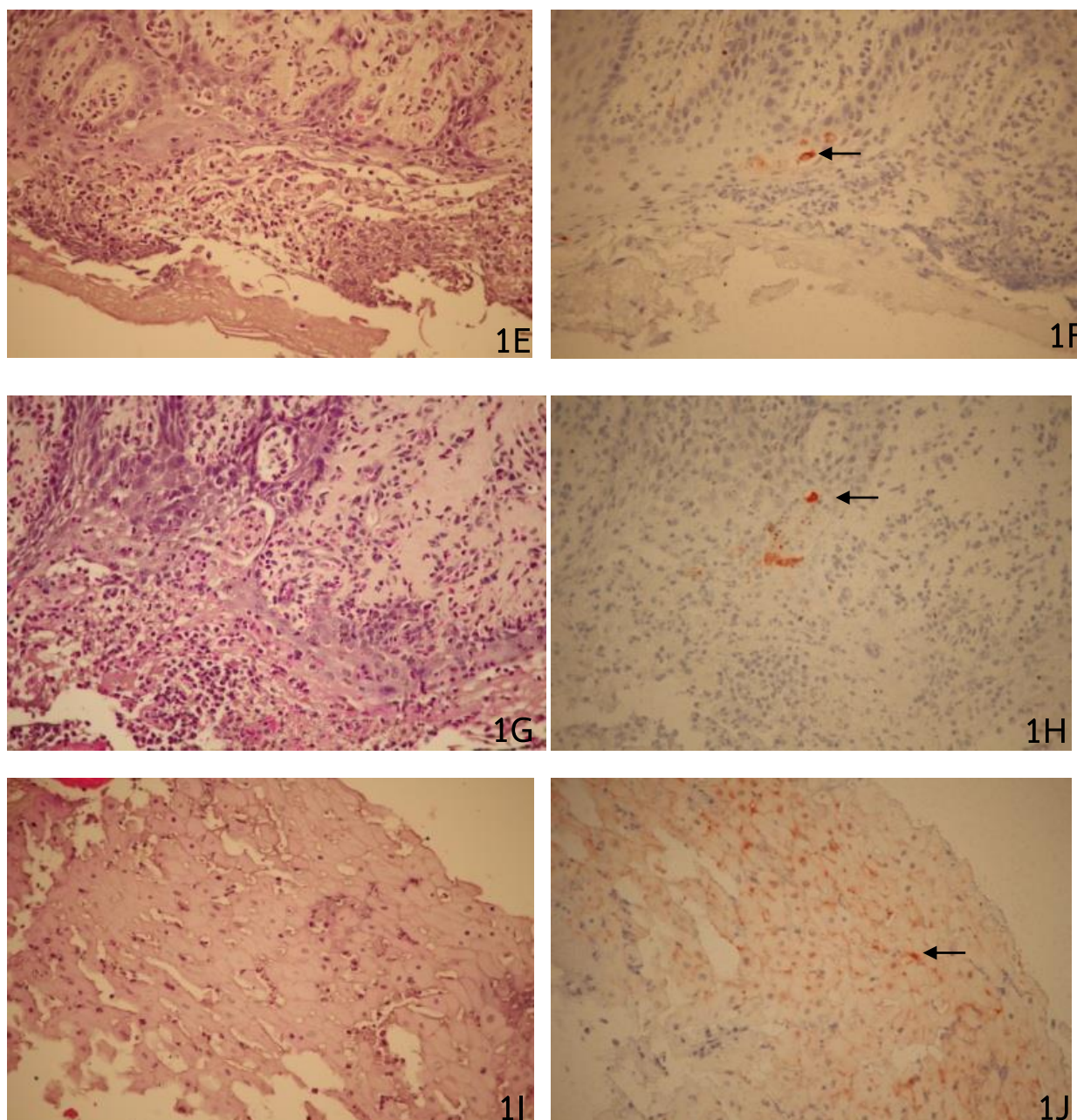
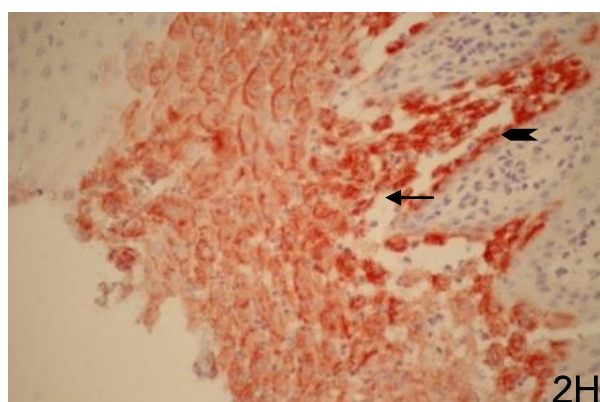
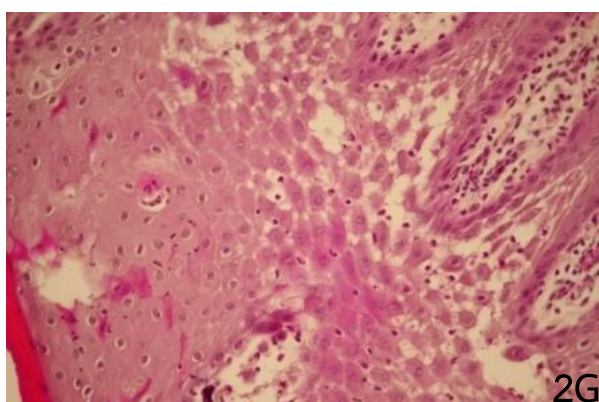
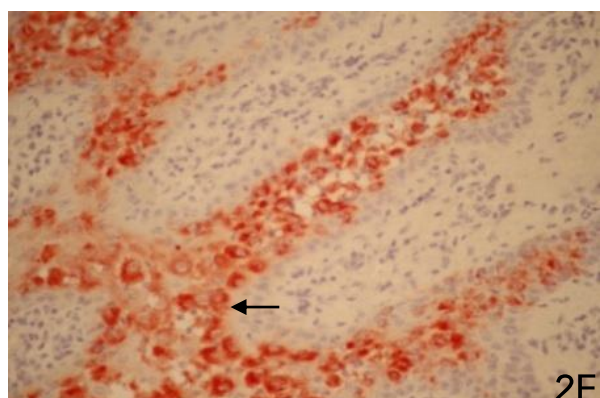
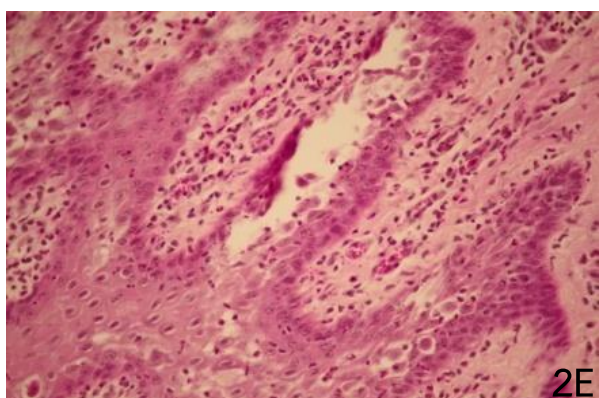
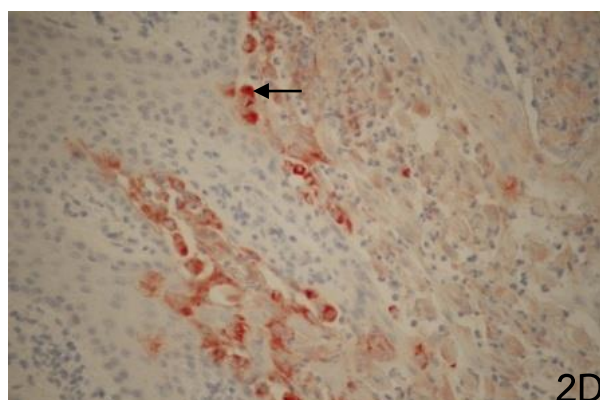
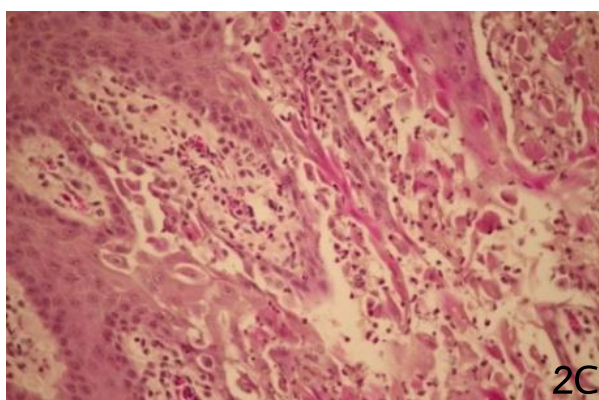
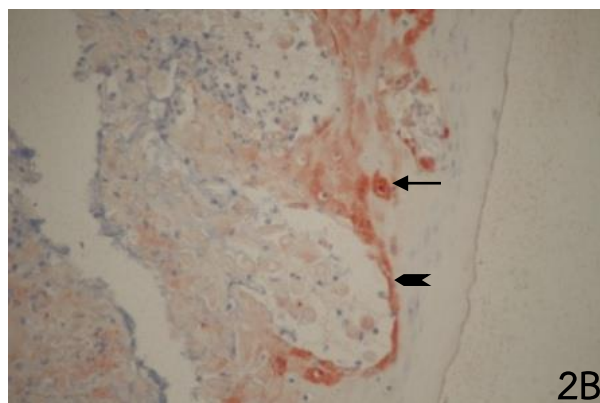
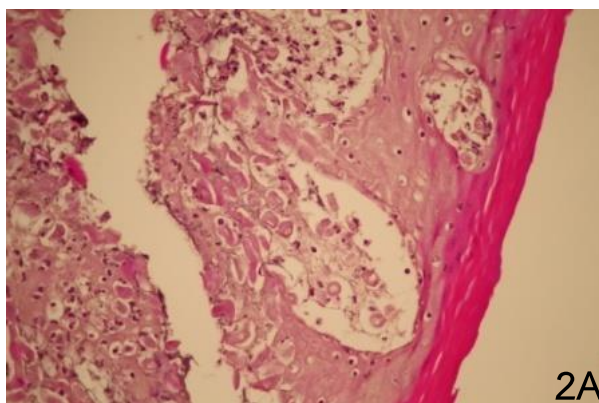


Figure 1. Tongue; FMDV type O strain O Udonthani/87 inoculated group. Presence of necrotic epithelium and inflammation of sub-epithelium stained by H&E original magnification 40x (Fig. 1 A, C, E, G and I for cattle no. 1, 2, 3, 4 and 5 respectively). IHC applied in the same area, FMDV type O antigen detected by mouse against FMDV type O monoclonal antibody (red color) in necrotic epithelial cells (←), Mayer's haematoxylin counterstained, original magnification 40x (Fig. 1 B, D, F, H and J for cattle no. 1, 2, 3, 4 and 5 respectively).







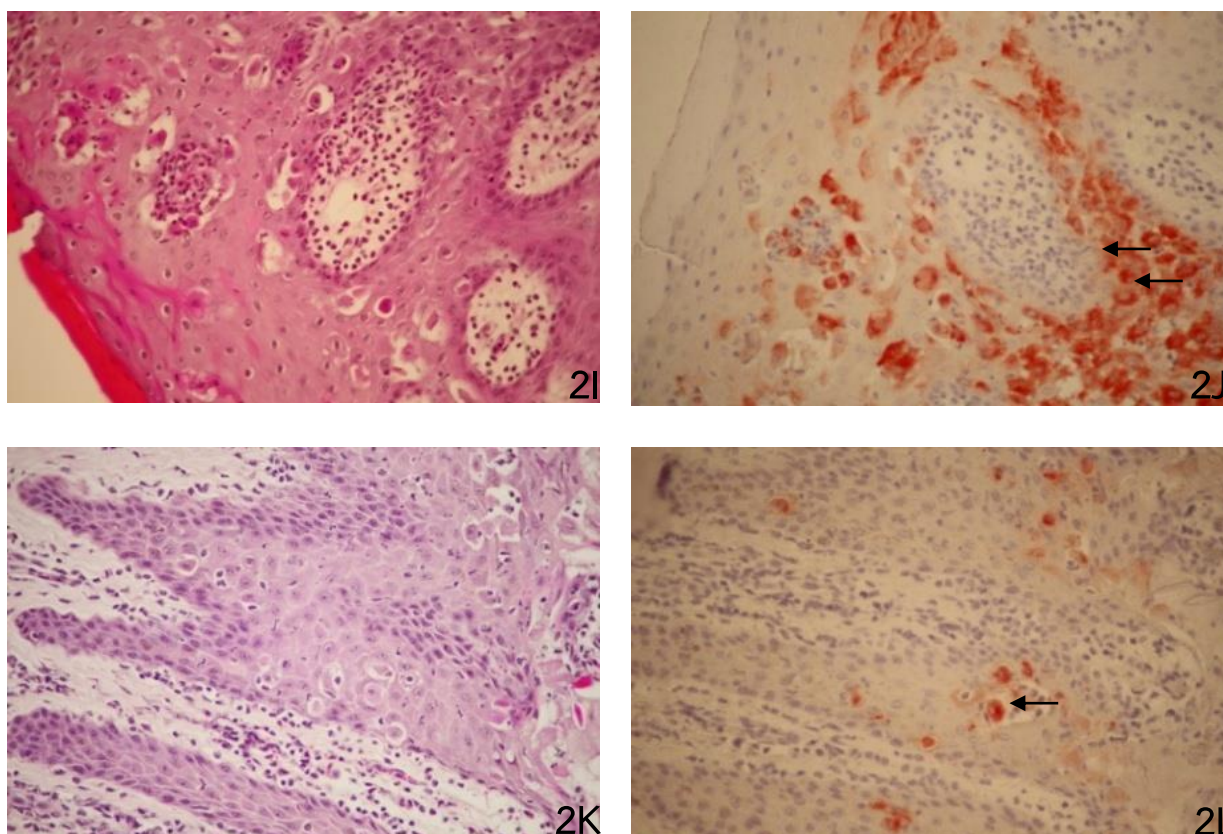


Figure 2. Interdigital skin; FMDV type O strain O Udonrthani/87 inoculated group. Presence of necrosis in epidermis and inflammation of dermis stained by H&E original magnification 40x (Fig. 2 A, C, E, G and I, for cattle no. 1; fig. 2 K for cattle no.3). IHC applied in the same area, FMDV type O antigen detected by mouse against FMDV type O monoclonal antibody (red color) in epithelial cells of the stratum spinosum (←) and stratum basale (◄), Mayer's haematoxylin counterstained, original magnification 40x (Fig. 2 B, D, F, H and J for cattle no. 1; fig. 2 L for cattle no.3).

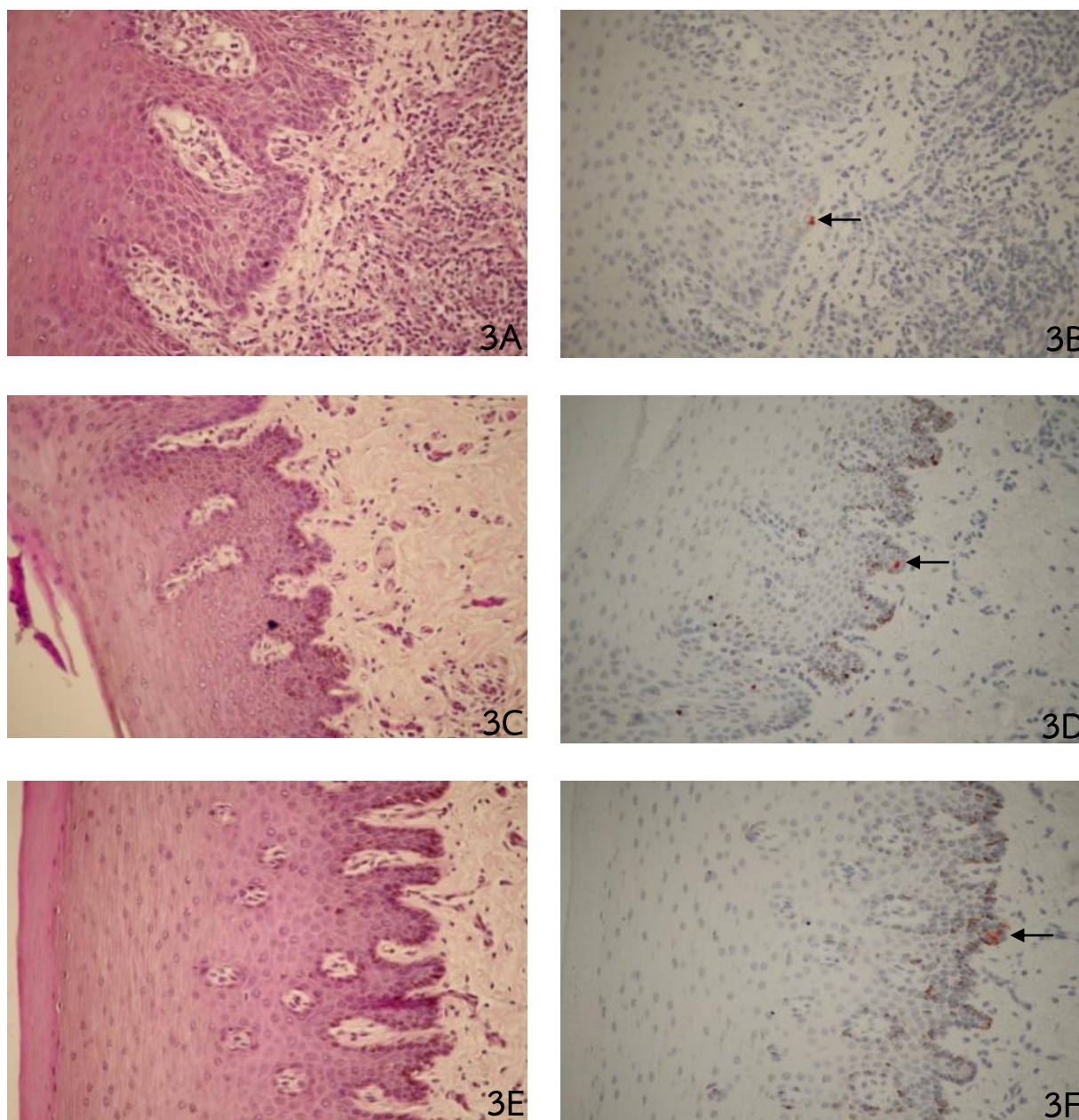


Figure 3. Dorsal soft palate of cattle no. 5; FMDV type O strain O Udonrthani/87 inoculated group. Presence of mild inflammation of sub-epithelium (Fig. 3 A) and no specific lesions (Fig. 3 C and E) stained by H&E original magnification 40x. IHC applied in the same area, FMDV type O antigen detected by mouse against FMDV type O monoclonal antibody (red color) in epithelial cells ( ← ), Mayer's haematoxylin counterstained, original magnification 40x (Fig. 3 B, D and F).

# การใช้เทคนิค Immunohistochemistry ในการตรวจหาเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอในเนื้อเยื่อโค

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## บทคัดย่อ

นำเทคนิค Immunohistochemistry (IHC) มาใช้ในการตรวจหาแอนติเจนของเชื้อไวรัสโรคปากและเท้าเปื่อย ไพบีโอ ในเนื้อเยื่อโคที่ผ่านการตรึงด้วยน้ำยาฟอมาลินและฝังในพาราฟิน เนื้อเยื่อดังกล่าวได้จากโคสุขภาพดีอายุ 6 เดือน จำนวน 5 ตัว ที่ได้รับการฉีดเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ (O Udonthani/87) เข้าที่ลิ้นตรงตำแหน่ง intradermolingual และเพื่อตรวจสอบปฏิกิริยาไม่จำเพาะต่อเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโออื่น ได้นำเนื้อเยื่อโคสุขภาพดีอายุ 6 เดือนที่ได้รับเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ (A Lopburi/2012) และไพบีโอเอเชียวัน (Asia-1 Nakornpathom/ 85) จำนวนไพบีโอละ 2 ตัว โดยฉีดเชื้อไวรัสโรคปากและเท้าเปื่อยวิธีเดียวกันกับไพบีโอ ร่วมทำการทดสอบ โดยใช้ mouse against FMDV type O monoclonal antibody เป็นไพรimaryแอนติบอดีในการตรวจจับแอนติเจนของเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ ผลพบปฏิกิริยาบวกเฉพาะในเนื้อเยื่อโคกลุ่มที่ฉีดเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ (O Udonthani/87) โดยพบปฏิกิริยาบวกในขนาดปานกลางถึงรุนแรงที่เซลล์บริเวณเนื้อตายและลอกหลุดของเยื่อบุลิ้น (5/5) และเนื้อเยื่อระหว่างกีบที่ชั้น stratum spinosum และ stratum basale (2/5) นอกจากนี้พบปฏิกิริยาบวกในขนาดรุนแรงน้อยในเซลล์เยื่อบุผิวของ dorsal soft palate (1/5) การเปลี่ยนแปลงทางจุลพยาธิวิทยาที่สำคัญ ได้แก่การอักเสบร่วมกับการลอกหลุดและเนื้อตาย ของเยื่อบุลิ้น (5/5) เยื่อบุเนื้อเยื่อระหว่างกีบ (3/5) เหงือก (2/5) และ larynx (2/5) และการอักเสบแบบเรื้อรังของ epiglottis (1/5) และ dorsal soft palate (1/5) จากการศึกษาในครั้งนี้พบว่าเทคนิค IHC ให้ผลดีในการตรวจหาแอนติเจนของเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ โดยมีความจำเพาะและไม่มีปฏิกิริยาข้ามกับเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโออื่นที่ได้ทำการศึกษาร่วม จากปฏิกิริยาที่พบแสดงถึงความสามารถของ mouse against FMDV type O monoclonal antibody ซึ่งเป็นสิ่งสำคัญในการตรวจจับแอนติเจนของเชื้อไวรัสทั้งที่มีปริมาณน้อยได้ ทั้งนี้เนื้อเยื่อสำคัญในการทดลองครั้งนี้ที่มีโอกาสพบแอนติเจนของเชื้อไวรัส ได้แก่เนื้อเยื่อที่มีรอยโรคเนื้อตายและลอกหลุดโดยเฉพาะเยื่อบุลิ้น และเนื้อเยื่อระหว่างกีบ สรุปได้ว่าเทคนิค IHC สามารถนำมาใช้เป็นทางเลือกในการตรวจหาแอนติเจนของเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอได้ เนื่องจากเป็นวิธีที่มีความไว ถูกต้องแม่นยำ และใช้เวลาเพียง 2 วันนับตั้งแต่เนื้อเยื่อได้ผ่านการตรึงด้วยน้ำยาฟอมาลินและฝังในพาราฟิน

**คำสำคัญ :** แอนติเจนของเชื้อไวรัสโรคปากและเท้าเปื่อยไพบีโอ, เนื้อเยื่อโค, เทคนิค IHC, ลิ้น, เนื้อเยื่อระหว่างกีบ