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Recommended Citation
Sunyasootcharee, Boonmee; Mutoh, Yuko; Tesaprateep, Ted; Tateyama, Susumu; Sailasuta, Achariya; and Yamaguch, Ryoji (1991) "Application of the immunohistochemical method for detection of Aujeszky's disease viral antigen in the field cases of hog cholera in Thailand," The Thai Journal of Veterinary Medicine: Vol. 21: Iss. 1, Article 3.
DOI: https://doi.org/10.56808/2985-1130.1565
Available at: https://digital.car.chula.ac.th/tjvm/vol21/iss1/3

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Application of the immunohistochemical method for detection of Aujeszky's disease viral antigen in the field cases of hog cholera in Thailand

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Application of the immunohistochemical method for detection of Aujeszky's disease viral antigen in the field cases of hog cholera in Thailand

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Abstract

Avidin-biotin-complex (ABC) method for Aujeszky's disease viral antigen was applied to cases suspected of hog cholera. Paraffin-embedded specimens of twenty-five such cases were retrieved. Aujeszky's disease viral antigen was detected in cerebrum (1/18), cerebellum (6/20), medulla oblongata (1/3) and tonsils (2/9). Totally, the viral antigen was found in eleven cases out of twenty-five. These results strongly suggested the possibility of concurrent infection of Aujeszky's disease and hog cholera.

Keyword(s): Aujeszky's disease, immunohistochemistry, Hog cholera

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Introduction

Experiences in Thailand have shown that under field conditions Aujeszky's disease does not only affect the newborn piglets in which discerned by the manifestation of nervous signs, but the disease does also afflict the two to three months old pigs in which the manifestation is but with only slight clinical symptoms. As a result of the subtle manifestation of Aujeszky's disease in older pigs it has become suspicious that the incident of the disease is likely to be obscured when it concurrently occurs with other diseases. Consequently, when concurrent infections occur, cases are diagnosed as of the disease which manifests the most vivid clinical symptoms, and Aujeszky's disease viral infection is frequently missed due to its subtle clinical signs. The possibility of a concurrent infections of Aujeszky's disease virus with other bacterial or viral pathogens such as hog cholera has already been suggested (Lai et al, 1984).

Hog cholera is a well-known viral disease caused by Togaviridae, pestivirus (Horzinek et al, 1973) which still remains endemic in Thailand. In contrast, the incident of hog cholera in Japan is just sporadic due to a more integrate vaccination program. Although vaccination with live-vaccine is also practiced in Thailand, it is incomplete in its extent and as a further complication in vaccine breakdown is frequently occured. Furthermore, hog cholera of less virulent strain that caused the mild chronic form pose problems in differentiating it from Aujeszky's disease. (Siegmund et al, 1973).

Immunohistochemical techniques are used to investigate the status of Aujeszky's disease viral infection from cases which had earlier on been histopathologically diagnosed as hog cholera.
Materials and Methods

Paraffin embedded specimens from twenty five cases that have clinically and histopathologically been diagnosed as hog cholera by the department of Pathology, Faculty of Veterinary Science Chulalongkorn University were prepared for routine histopathology and immunohistochemistry in the same manner as previously described (Mutoh et al, 1991).

The tissues provided for this study were cerebra from eighteen cases, cerebella from twenty cases, medulla oblongata from three cases and tonsils from nine cases. No spinal cord was provided for the present study. In addition, tissues such as kidney, lung were included in the submitted blocks were also examined occasionally. Tissue sections were stained with haematoxylin and eosin (H&E), and Avidinbiotin-complex (ABC) method was also applied as previously described by Mutoh et al, (1991).

Results

Histopathological observation revealed that specimens that have been diagnosed as hog cholera with non-suppurative encephalitis exhibited more severe lesions of pan-encephalitis when compared with specimens diagnosed as Aujeszky's disease. Nerve cells were more karyopyknosis. In the cerebellum, the granular cell layer occasionally revealed necrosis (2/15) but most of them appeared normal. Tonsils were normal, only tonsilar abscess with pus accumulation and bacterial clump in crypts were occasionally observed. Endothelium that lined the blood vessels was also damaged. Immunohistochemical examination for Aujeszky's disease viral antigen in hog cholera suspected cases demonstrated positive reaction in eleven cases out of twenty-five.
Fig. 1: Positive nerve cell (arrow) in cerebellum observed in hog cholera case. ABC. x400.

Fig. 2: Positive Purkinje cell (arrow) in hog cholera case, cerebellum ABC. x400.
The specimens that showed positive reaction were one cerebrum out of eighteen, eight cerebella out of twenty and one medulla oblongata out of three. The positive reaction was observed in the nerve cells and glial cells of cerebrum and cerebellum, and also Purkinje cells in the cerebellum (Figs. 1,2). Although both the cerebrum and cerebellum revealed positive reaction, the reaction was more frequently examined in the cerebellum.

The tonsils appeared normal and revealed non-viral necrosis on H&E staining, however, basal cells of the crypts epithelium and lymphocytes revealed positive staining in two cases out of nine. Necrotic area exhibited no reaction to Aujeszky’s disease viral antigen.

In other tissues, tubuli of one kidney specimen revealed positive reaction while the specimens of central nervous system and tonsils from the same case revealed only weak reaction. One lung and lymph node also showed positive reaction (Table 1).

Discussion

Some of the cases which were demonstrated as positive for the Aujeszky’s disease viral antigen had previously been shown to reveal the characteristic macroscopic features of hog cholera such as generalized skin hemorrhages, strawberry lymph nodes, Turkey egg of kidney, and button ulcer in the intestine (Stewart et al, 1982). These results strongly suggested the possibility of concurrent infection of Aujeszky’s disease and hog cholera.

The pigs which are suggested as cases of concurrent infection of Aujeszky’s disease and hog cholera in the present study, had not previously been considered as concurrently suffering from the Aujeszky’s disease viral infection. Upon observation some additional tissues, presence of viral antigen in tubuli of the
Table 1: Distribution of Aujeszky’s disease Viral Antigen on tissue sections of hog cholera suspected cases.

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Organs</th>
<th>Central Nervous System</th>
<th>Tonsil</th>
<th>Others*</th>
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<tbody>
<tr>
<td></td>
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<td>Cerebrum</td>
<td>Cerebellum</td>
<td>Medulla oblongata</td>
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* Other organs with positive reaction
+ Positive
- Negative
† Weak positive
* No specimens were taken
kidney was observed. Desquamation of tubular epithelial cells would result in the release of viral antigen into the urine (Ducatelle et al, 1982). In support of this, McFerran et al, (1964) reported that Aujeszky’s disease virus has previously been isolated from pig urine.

In the present study, only a few pieces of specimens had been prepared and if the specimens from the central nervous system and other organs could be prepared more carefully, the feasibility of the study of the ABC method might have been demonstrated with an even higher efficiency.

The ABC method proved to have a clear benefit as a technique for retrospective studies. However, the ABC method has a limitation in that it can not detect the latent infection. To overcome this limitation, in situ hybridization, a method which can detect latent infections has been developed (Olander et al, 1966). This new method would however be an outlet in an endeavor aiming at the eradication of Aujeszky’s disease.

Acknowledgement

The authors are gratefully acknowledging the Ministry of Education, Science and Culture of Japan for supporting this work by a Grant-in-Aid for Scientific Research (No. 01045027), and Dr. Masuo Sueyoshi of the National Institute of Health, Kagoshima for providing the anti-Aujeszky’s disease virus rabbit antiserum.
References


การตรวจหาอจจุบันวัสดุแอนติเจนในสมอง...

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บทคัดย่อ

ให้ทำการตรวจหาวัสดุแอนติเจนในสมองเนื้อเยื่อที่พิษภัยในพืชและสัตว์ทั่วไปกับวัสดุแอนติเจนซึ่งที่มีผลที่สำคัญในงานวิจัยที่นับ 25 ราย โดยมีวัสดุแอนติเจนในสิ่งต่างๆ ใช้อาทิตย์-โปรตีนคอมแพล็กซ์ (Avidin-biotin Complex) ตรวจพบว่ามีวัสดุแอนติเจ็นในสมองส่วนกลาง (cerebrum) 1 ใน 18 ราย, สมองหน้า (cerebellum) 6 ใน 20 ราย, เบ็ดท่อหลังสงคราม (Medulla oblongata) 1 ใน 3 ราย, และคอหู (Tonsils) 2 ใน 9 ราย ที่สำคัญของการตรวจหาวัสดุแอนติเจ็นพบ 11 ราย ใน 25 รายของสัตว์ที่มีวัสดุแอนติเจน ผลการศึกษาความเชื่อมโยงที่น่าสนใจความบ่งชัดในการคัดแยกความเป็นที่อยู่ของวัสดุแอนติเจ็นและวัสดุแอนติเจ็น

ค่าสำคัญ : วัสดุแอนติเจนวัสดุแอนติเจน, อิมมูโนซิโลแมส, โรคคล้วยภาษา

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