

## PITUITARY ADENOMA IN A HORSE: A CASE REPORT

Nopadon Pirarat<sup>1\*</sup> Kohsaku Chijiwa<sup>2</sup> Pattama Rittruechai<sup>3</sup>  
Sawang Kesdangsakonwut<sup>1</sup> Komkrich Teankum<sup>1</sup> Wijit Banlunara<sup>1</sup>  
Anudep Rungsipipat<sup>1</sup> Boonmee Sunyasutjaree<sup>1</sup>

### Abstract

Nopadon Pirarat<sup>1\*</sup> Kohsaku Chijiwa<sup>2</sup> Pattama Rittruechai<sup>3</sup>  
Sawang Kesdangsakonwut<sup>1</sup> Komkrich Teankum<sup>1</sup> Wijit Banlunara<sup>1</sup>  
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A female, grey, 24-year-old horse was showing clinical signs of weight loss, ataxia, hersutism, high fever, dysuria, nasal discharge and moist rale lung sounds. Gross examination revealed a single, white, firm, pituitary mass with a diameter 2.5 x 2.1 x 1.5 cm, compressed and overlying the hypothalamus. Histopathological and immunohistochemical examinations revealed the characteristic of a ACTH-derived pituitary adenoma.

**Keywords :** horse, pituitary adenoma, immunohistochemistry

<sup>1</sup>Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330.

<sup>2</sup>Department of Veterinary Pathology, Faculty of Agriculture, Miyazaki University, 889-2192 Japan.

<sup>3</sup>Faculty of Veterinary Medicine, Mahidol University, Nakhon Prathom. 73170

\*Corresponding author

<sup>1</sup>ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

<sup>2</sup>ภาควิชาพยาธิวิทยาทางสัตวแพทย์ คณะเกษตรศาสตร์ มหาวิทยาลัยมิยาซากิ ญี่ปุ่น 889-2192.

<sup>3</sup>คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล นครปฐม 73170

\*ผู้รับผิดชอบบทความ

## บทคัดย่อ

นพดล พิพารัตน์<sup>1\*</sup> โภคากุ ชิจิ瓦<sup>2</sup> ปัทมา ฤทธิฤทธิ์ชัย<sup>3</sup> สว่าง เกษยแดงสกกลวุฒิ<sup>1</sup> คมกฤษ เทียนคำ<sup>1</sup> วิจิตร บรรลุนารา<sup>1</sup> อนุเทพ รังสีพิพัฒน์<sup>1</sup> บุญมี สัญญาสุจารี<sup>1</sup>

### รายงานสัตว์ป่วย: เนื้องอกของต่อมใต้สมองในม้า

ม้าเพศเมียอายุ 24 ปีแสดงอาการทางประสาท ไข้สูง น้ำหนักลด ขนขี้นยวบเร็วผิดปกติ (hirsutism) ปัสสาวะลำบาก มีน้ำมูกและเสียงปอดผิดปกติ ผลการหันสูตรพบก้อนเนื้อสีขาวบริเวณต่อมใต้สมอง มีลักษณะเหมือนขนาดประมาณ  $2.5 \times 2.1 \times 1.5$  ซม. การตรวจทางจุลทรรศน์วิทยาและอัมมูโนอิมมูโนซีโตเคมี วินิจฉัยก้อนเนื้อที่พบเป็นเนื้องอกของต่อมใต้สมองชนิดที่สร้างฮอร์มอน ACTH

คำสำคัญ: ม้า เนื้องอกของต่อมใต้สมอง อัมมูโนอิมมูโนซีโตเคมี

### Introduction

Pituitary tumors are rare in animals and horses, are mainly derived from pars intermedia cells. Pituitary adenoma usually develops in older horses and more frequently in females (Boujon et al., 1993). Adenomas of the pars intermedia are usually large neoplasms that extend out of the sella turcica and severely compress the overlying hypothalamus. A sharp line of demarcation remains between the neoplasm and the compressed pars distalis.

The clinical signs of hirsutism, hyperhidrosis, polydipsia, polyphagia, insulin resistant hyperglycemia, polyuria, glycosuria and increased susceptibility to infectious diseases are usually manifested (Eiler et al., 1997). Equine pituitary adenoma syndrome is related to the over production of several pro-opiomelanocortin (POMC)-derived peptides by the tumor cells and does not correspond to human Cushing's disease.

The tumor in horses secretes not only adrenocorticotrophic hormone (ACTH) but pro-opiomelanocortin, (POMC)-derived peptides, such as (- and  $\beta$ -melanocyte-stimulating hormone ( $\alpha$ - and  $\beta$ -MSH), corticotrophin-like intermediate lobe peptide and  $\beta$ -endorphin ( $\beta$ -END) (Wilson et al., 1982; Beech and Marolo, 1985).

Pituitary adenomas in horses were first reported in 1932 (van der Kolk et al., 1993), however the tumor has never before been reported in Thailand. This present study reports on a pituitary adenoma in a horse and characterizes the equine pituitary adenoma by clinical, histopathological and immunohistochemical studies.

### Materials and Methods

A female, grey, 24 year-old horse had shown clinical signs of weight loss and ataxia for

2 months. Hersutism, high fever, dysuria, nasal discharge and moist rale were manifested although the appetite had been normal. The horse was submitted to the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University for necropsy. The samples were fixed in 10% formalin, conventionally processed and embedded in paraffin. The sections were cut from paraffin blocks and stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) (Carson, 1997) for histopathological examination.

Immunohistochemical analysis using 1:100 rabbit polyclonal, anti-human adrenocorticotropic hormone (ACTH) antibody (DAKO, Japan) as the primary antibody were done at 4°C overnight. The sections were incubated with a secondary antibody-labeled, Polymer HRP, anti-mouse and anti-rabbit antibody (Envision Polymer/Dako, Japan) which develops a brown color by using the substrate of 3, 3' diaminobenzidine tetrahydrochloride (DAB) solution. They were counterstained with Meyer's hematoxylin.

## Results

A complete blood count revealed a moderate leucocytosis (20,850 cells/ul), mature neutrophilia (18,973 cells/ul), lymphopenia (1,876 cells/ul) and eosinopenia (0 cells/ul). Serum biochemistry showed an elevation of alanine aminotransferase (ALT), 100 units but normal aspartate aminotransferase (AST), 120 units. Creatinine was 1.1 mg%, blood urea nitrogen (BUN) 20 mg%, glucose 104 mg%, alkaline phosphatase 141 units, triglyceride 97

units, cholesterol 60 mg% and cortisol 3.4 µg/dl which were all within the normal range (Eades and Bounous, 1997).

Macroscopic findings showed a single, white, firm mass with a diameter about 2.5 x 2.1 x 1.5 cm extending out of the sella turcica area and compressing the overlying hypothalamus (Figure 1). The cut surface revealed a dark rose color at the center of the mass.

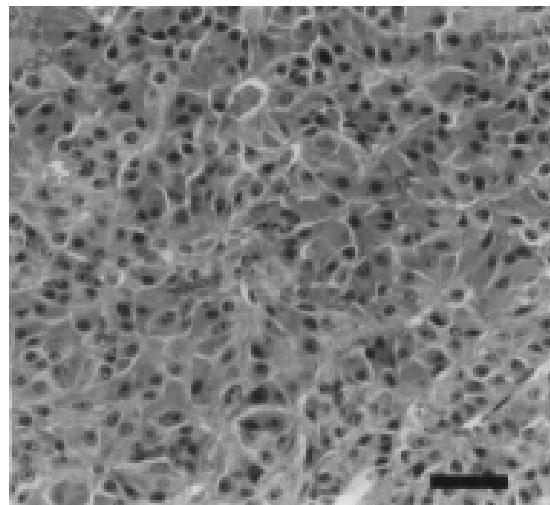
Microscopically, the pituitary mass was well delineated and located confluent with the pars intermedia. The boundaries with the pars nervosa and pars distalis were well demarcated. The mass was composed of nests and cords of neoplastic cells separated by a moderate fibrovascular stroma with infrequent formation of large blood-filled sinusoids (Fig 2). The neoplastic cells were polyhedral to elongated, with distinct cytoplasmic boundaries and a moderate amount of finely granular, faintly eosinophilic, cytoplasms and ovoid hyperchromatic nuclei. Mitotic figures and pleiomorphic nuclei were occasionally seen. In some areas, a palisading pattern of tumor cells was apparent along the fibrovascular septa. Hemorrhage, hematoidin pigment and macrophages, loaded with hemosiderin pigment, were frequently observed.

PAS staining showed a characteristic pattern of glandular or adenomatous lesions with homogeneous, pink, luminal secretions. Numerous tumor cells contained homogeneous, pink, positive reactions in their cytoplasms and luminal secretions (Fig 3).

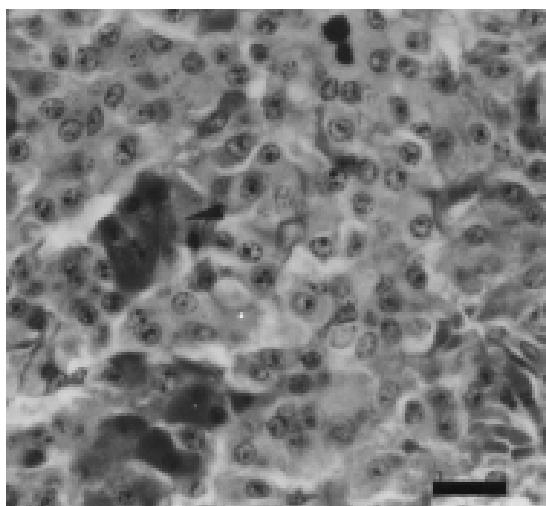
Immunohistochemical examination using a polyclonal, anti-ACTH, antibody revealed a



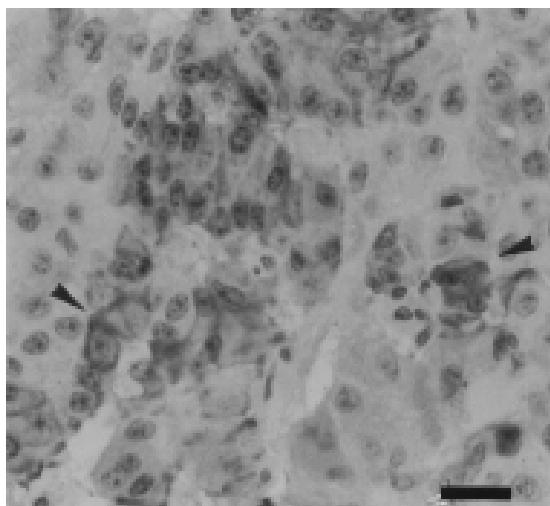
**Figure 1.** Pituitary adenoma arising from the pars intermedia.



**Figure 2.** Pituitary adenoma from the pars intermedia displaying neuroendocrine architecture and rosette formation of neoplastic cells around the capillaries (H&E, bar = 50  $\mu$ m).



**Figure 3.** Numerous tumor cells contained homogeneous, pink, positive reactions in their cytoplasms (PAS, bar = 25  $\mu$ m).



**Figure 4.** Positive immunoreactivity characterized by a dark brown, granular appearance in the cytoplasm of tumor cells (IHC, anti-ACTH, bar = 25  $\mu$ m).

positive reaction which was characterized by a dark brown granular appearance in the cytoplasm (Fig 4). No positive reaction could be observed in the luminal secretion of the neoplastic glandular structure.

## Discussion

In this present study, the gross and histopathological structure of the pituitary adenoma in a horse, involving the pars intermedia, was virtually identical to others that have been described (Boujon et al., 1993; van der Kolk et al., 1993; Okada et al., 1997). Microscopic features included a massive growth of basophilic cells, separated from each other by a delicate fibrous stroma and frequently seen small, lumina filled with colloid material of a typical neuroendocrine architecture (van der Kolk et al., 1993). There is a distinct difference between the disease in horses and humans; in horses, ACTH is secreted from the cells of the pars intermedia but in humans, it is secreted from cells in the pars distalis. In dogs with hyperadrenocorticism, ACTH is secreted in the pars distalis and pars intermedia (Okada et al., 1997). Plasma cortisol is not a good indicator of pituitary tumors in horses, unlike in humans or dogs. Many investigations reviewing plasma cortisol concentrations in healthy horses gave variable results and showed diurnal fluctuations with concentrations highest in the morning (Garcia and Beech, 1986; Horvath et al., 1988). Horses with a pituitary adenoma frequently have a normal, complete blood count and blood chemistry panels. However, increased neutrophil counts,

decreased lymphocyte and eosinophil counts are common because of hypercortisolemia. Plasma cortisol concentration is infrequently increased in horses with a pituitary adenoma (Eades and Bounous, 1997)

Although many of the functional disturbances in horses with a pituitary adenoma, such as, diabetes insipidus, polyphagia, hyperpyrexia, hyperhidrosis, or hirsutism, appear to be the result of hypothalamic or neurohypophyseal dysfunction, other behavioral signs such as diminished responsiveness to painful stimuli, can be related to increased plasma and cerebrospinal fluid levels of beta-endorphin. The clinical symptoms in horses are distinctly different from that of Cushing's disease in dogs, cats and human patients. Hirsutism, an excessively long and curly coat, often relates to a history of failure to shed hair. A pituitary adenoma is the only clinical condition known to cause hirsutism in the horse (Sweeney et al., 1989). The exact mechanism for hirsutism in the horse is unknown, the excessive secretion of melanocyte-stimulating hormone may play an important role.

Immunohistochemistry showed that the pituitary adenoma originated from adrenocorticotrophic hormone secreting cells of the pars intermedia. According to other investigators, there are several immunohistochemical markers for the characterization of pituitary adenoma in horses including ACTH,  $\beta$ -lipotropin,  $\beta$ -END,  $\alpha$ -MSH and  $\beta$ -MSH (Boujon et al., 1993; Okada et al., 1997; Mendez et al., 1998). Immunoreactivity with several kinds of antibodies and overproduction of different POMC-derived peptides suggests that the pituitary,

adenoma-dependent, equine syndrome is different from Cushing's disease in other species (Boujon et al., 1993; Mendez et al., 1998).

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