Peripheral Neuroblastoma and Primitive Neuroectodermal Tumor in Japanese Black Cattle

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ABSTRACT. Peripheral neuroblastoma was found in a 1-year-old, male, Japanese black cattle (Case 1) and primitive neuroectodermal tumor was noted in 7-year-old, female, Japanese black cattle (Case 2). In Case 1, neoplastic tissue was replaced the right cranial vault and nasopharynx. A large, soft mass approximately 18 cm in diameter was also observed in the right mandibulopharyngeal area. In Case 2, a neoplastic mass of about 15 cm in diameter was found in the mandibulopharyngeal area. Histopathologically, massive necrosis showing a pseudopalisade arrangement was frequently observed in Case 1. On the contrary, Homer & Wright rosette formations of tumor cells were prominent in Case 2. Immunohistochemically, the proliferating cells in Case 1 were positive for vimentin, S-100, and neurofilament (NF) and those in Case 2 showed intense immunoreactivity for NF and neuron specific enolase, but were negative for vimentin and S-100. The different degrees of differentiation of the neoplastic cells originating from the neuroectoderm, might be reflected in their different morphological and immunohistochemical features. — KEY WORDS: bovine, peripheral neuroblastoma, primitive neuroectodermal tumor (PNET).

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Neuroectodermal tumors, neoplasms derived from primitive neuronal cells, in animals are rigorously classified [5], into neuroblastomas, ganglioneuroblastomas, and ganglioneuromas according to the degree of differentiation of the neoplastic cells [3, 6, 15]. In general, neuroblastomas including medulloblastomas in the central nervous system, are very rare in domestic animals [6]. In the peripheral nervous system, few cases of neuroblastomas in the adrenal medulla, sympathetic ganglia, or nasal cavity (aesthesioneuroblastomas) have been reported in several animal species [1, 3, 4, 6, 11, 14]. Cordy [3] reported that peripheral neuroblastomas analogous to human *in situ* neuroblastomas, were often found in adult slaughter cattle and malignant neuroblastomas were seen in premature or stillborn calves and in dogs of all ages [6].

Recently, very primitive tumors with a potential to differentiate into both neuronal and glial cells have been called primitive neuroectodermal tumors (PNETs) in the medical field [8–10, 15–17]. The term PNETs has been applied to poorly differentiated tumors both in the central and peripheral nervous system, but the term has not been well-generalized to tumors of domestic animals [17, 18]. Although the PNET was originally coined to designate unclassifiable tumors consisting of undifferentiated neuroectodermal cells with variable neuro-glial differentiation, recently the term is sometimes applied to conceptually to all embryonal neuroectodermal tumors including medulloepitheliomas, medulloblastomas or neuroblastomas [8–10]. However, several pathologists [8–10] proposed that the diagnosis of PNET should be applicable only in instances wherein no differentiation was detected by morphological examinations, or in instances wherein various differentiation was observed and the lesions did not fit neatly into a recognized diagnostic category.

Immunohistochemical examination of neuroblastomas or PNETs has been attempted for several human cases [7, 12, 13, 15, 19]. In general, human neuroblastomas show immunoreactivity for neurofilament (NF), and some exhibit positive reactions for S-100, neuron specific enolase (NSE), chromogranin A, or synaptophysin [15, 19] although the immunoreactivity varied among cases. In addition, tumor cells with squamous differentiation are sometimes positive for keratin or cytokeratin [15]. In animals, similar investigation have been done in a few cases [11] and there are few informations concerning the immunohistochemical natures of animal neuroblastomas or PNETs [18].

The present paper describes morphological and immunohistochemical natures of two bovine tumors supposed to be originated from the neuroectoderm and discusses their differences. The histopathological designations of these tumors are applied according to the recent human WHO classification of nerve tumors discussed by Okazaki [9]

Case history:

Case 1: The animal was a Japanese Black bull, born on April 4, 1994 in Miyazaki, Japan. He showed neurological signs with abnormal movement of the right eye in January 1995. From these neurological symptoms, the animal was presumed to have an infectious disorder of the nervous system and was treated with several antibiotics. The clinical sign did not improve and the disease continued to progress. On April 4, 1995, he exhibited astasia, exophthalmos of the right eye, fracture around the right horn, and severe swelling of the right side of the face and head. X-ray examinations of the head revealed a neoplastic mass replacing the right cranial vault or nasopharynx, but not the nasal cavity. The bull was euthanized on the next day and an autopsy was performed immediately in our laboratory.

Case 2: This animal was a 7-year-old Japanese Black cow. The cow showed severe dyspnea and was clinically

diagnosed as having acute pneumonia. However, the details of the clinical history of this case could not be confirmed. The cow was killed and an autopsy was performed in September, 1995 at the Miyazaki Prefecture Meat Inspection Laboratory.

Histological procedures:

For routine histopathology, tissue samples were fixed with 10% neutral buffered formalin. The samples from Case 1 were also fixed with methanol-Carnoy's solution for immunohistochemical examination and with 2% glutaraldehyde for electron microscopy. Paraffin sections were stained with hematoxylin and eosin (HE) and Watanabe's silver impregnation. For electron microscopy, glutaraldehyde-fixed samples from Case 1 were post-fixed with 2% osmium tetroxide and embedded in epoxy-resin. Immunostaining was performed using a kit employing the avidin-biotin peroxide complex (ABC) method (Vectastain PK-4000, Vector Laboratories, Burlingame, CA, U.S.A.). Before sections from Case 2 were immunostained, hydrated autoclave pretreatment was performed. The primary antibodies used were monoclonal antibodies against bovine NF-200 (1:20, Transformation Research Inc., Framingham, MA, U.S.A.), bovine NF-145 (1:20, Transformation Research Inc.), bovine NF-68 (1:20, Transformation Research Inc.), synaptophysin (1:10, Boehringer Mannheim, Mannheim, Germany), and vimentin (1:10, Boehringer Mannheim), as well as rabbit antisera against S-100 (1:200, Dako, Carpinteria, CA), glial fibrillary acidic protein (GFAP, prediluted, Dako), and NSE (prediluted, Dako). The secondary antisera were biotinylated goat antisera against mouse immunoglobulins (1:200, Dako) and rabbit immunoglobulins (1:200, Dako).

Gross findings:

Case 1: The neoplastic mass was found to have replaced the right cranial vault and nasopharynx with disintegration of the surrounding bone. The mass extended to the endocranium of the right cerebral cortex and to the leptomeninges of the medulla of the 5th and 7th cranial nerves. A large, soft mass approximately 18 cm in diameter was also found in the right mandibulopharyngeal area (Fig. 1). The cut surface of this mass was gray to white. Severe necrotic foci with apparent hemorrhages were also observed. In the lungs, dark-red masses approximately 0.5 to 1.0 cm in diameter, were found in almost all the lobules. In addition, a white neoplastic mass, about 1.0 cm in diameter was observed on the endocardium of the right heart ventricle. No abnormalities other than these neoplastic changes were apparent.

Case 2: A large, hard mass approximately 15 cm in diameter, was found in the right mandibulopharyngeal area (Fig. 2). The cut surface of the mass was while and lobular in appearance. Metastatic lesions in the lymph nodes surrounding the neoplastic mass were found. In the other visceral organs, there were no significant lesions.

Histopathology:

Case 1: The neoplastic mass in the right cranial vault, nasopharynx, and mandibulopharyngeal area consisted of proliferating small tumor cells and severe necrosis with hemorrhage. These tumor cells were arranged in lobules and broad sheets or ribbon-like structures. Severe necrotic foci surrounded by pseudopalisade arrangements of neoplastic cells or their debris were apparent (Fig. 3). Most of the tumor cells were small and round with eosinophilic cytoplasm and atypical round nuclei containing abundant chromatin (Fig. 4). Granular deposits of calcium were frequently seen in the necrotic foci. Mitotic neoplastic cells were very common. Stromal connective tissue was not abundant, whereas reticulum fibers surrounding packs of neoplastic cells were prominent by Watanabe's silver staining. The 5th and 7th cranial nerves had almost been replaced by a proliferation of neoplastic cells, although the neoplastic tissues had not invaded the brain. In the lungs and heart, metastatic lesions with or without necrotic foci were seen. The morphological characteristics of these neoplastic cells were almost identical to those in the right cranial vault, nasopharynx, and mandibulopharyngeal area. In the other visceral organs, there were no significant lesions. Electron microscopy showed that the neoplastic cells appeared to have intermediate filaments and a few cytoplasmic organelles such as mitochondria and rough endoplasmic reticulum. There were no evidence suggesting some specific differentiation of the tumor cells.

Case 2: The neoplastic mass in the mandibulopharyngeal area consisted of a solid proliferation of small cuboidal to round cells sometimes forming cord-like structures (Fig. 5). The tumor cells were characterized by clear cytoplasm and round hypochromatic nuclei with distinct nucleoli. These tumor cells were sometimes arranged in tubular or adenoid structures called as Homer & Wright rosette (Fig. 6). In the stromal area, abundant reticulum fibers surrounding packs of these neoplastic cells were observed. Small necrotic foci were sometimes observed, but pseudopalisade cellular arrangement was not seen.

Immunohistochemistory:

Case 1: The neoplastic cells exhibited intense immunoreactivity for antibodies against vimentin (Fig. 7a), S-100, and bovine NF-200 (Fig. 7b). Almost all tumor cells commonly showed intense positive reaction for vimentin, S-100, and NF, while a subset of cells lacked NF-immunoreactivity. There was no significant immunoreactivity of tumor cells for the other antibodies.

Case 2: The tumor cells showed intense immunoreactivity for bovine NF-200 (Fig. 8a) and NSE (Fig. 8b). Both NF- and NSE- immunostaining recognized intensely the cytoplasm of neoplastic cells and stromal area within rosettes. Although the tumor cells were negative for S-100 and vimentin, small population of S-100- and vimentin-positive cells thought to be Schwann cells, were observed in the periphery of small round neoplastic cells forming Homer & Wright rosettes.

Since the cranial vault and nasopharynx were severely involved with tumor in Case 1, there is a possibility that neoplastic cells were derived from olfactory sensory cells called as olfactory neuroblastoma [1, 4, 14, 17]. However,

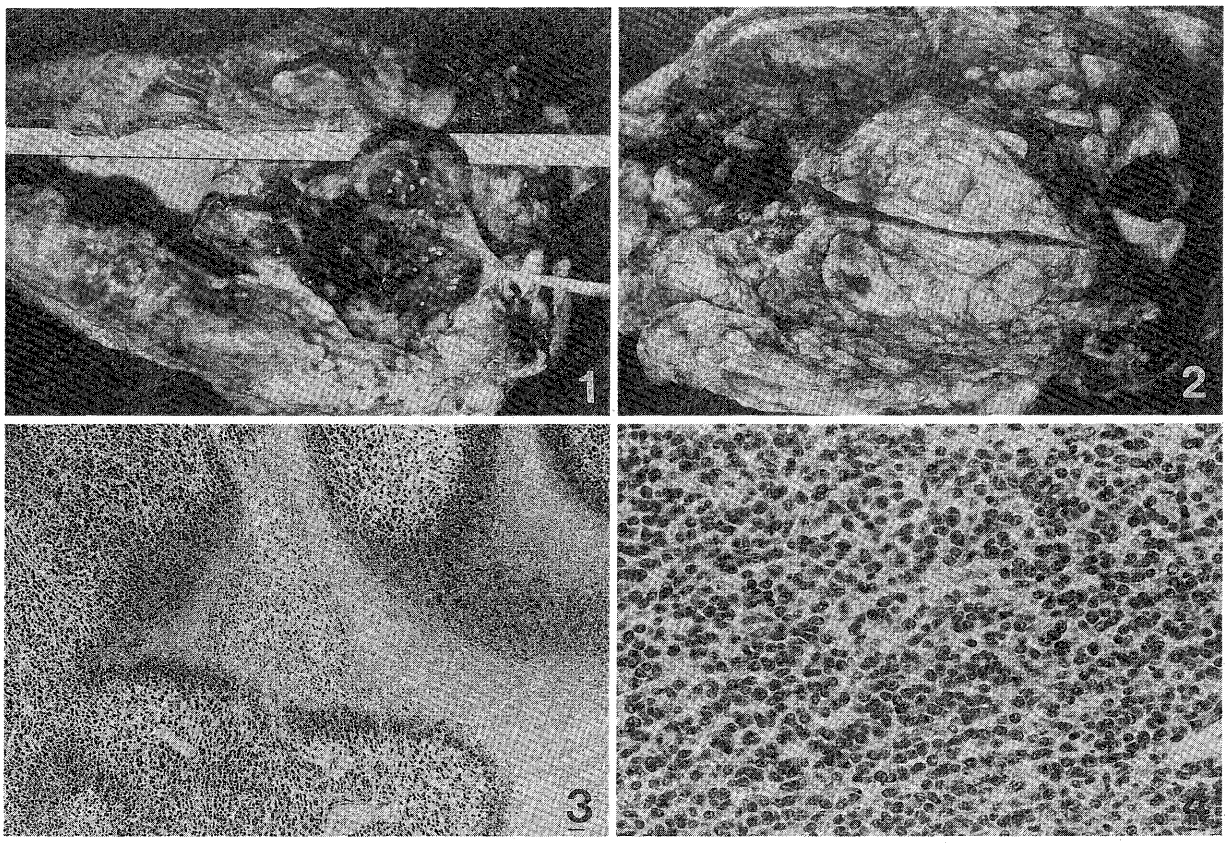

Fig. 1. Large, soft mass, approximately 18 cm in diameter, found in the right mandibulopharyngeal area. Case 1.
Fig. 2. Large mass, approximately 15 cm in diameter, found in the right mandibulopharyngeal area. The cut surface of the mass is white and lobular in appearance. Case 2.
Fig. 3. Severe necrotic foci with pseudopalisade arrangement of neoplastic cells. Case 1. HE. × 25.
Fig. 4. The tumor cells are small round cells with eosinophilic cytoplasm and hyperchromatic round nuclei. Case 1. HE. × 180.

the distribution of the tumor mass in Case 1 is quite different. The primary site is difficult to discern in a rapidly spreading malignant neuroblastoma [3], but we believe that the present tumors may have arisen from the ganglion or remaining neural crest cells in the peripheral nerve, and not from olfactory sensory cells.

The histological characteristics in Case 1, such as severe necrosis with pseudopalisade cellular arrangement and metastases show the malignancy of the tumor. Although a pseudopalisade structure is one of the characteristic lesions of glioblastomas and other malignant glial tumors including malignant schwannoma [15–17], any significant evidences of specific differentiation to glial cell were not found in case 1 and the tumor was supposed to be undifferentiated or very primitive tumors originating from the neuroectderm. In contrast, Homer & Wright rosette formations which are thought to be one of the characteristic lesions of neuroblastomas [15] were found in Case 2. Thus, Case 2 was diagnosed as a typical neuroblastoma.

The positive immunoreactivity of the neoplastic cells in both cases for NF and the negative reaction for GFAP suggest that these tumors have an potential to differentiate for neuronal cell. The tumor cells in Case 1 were also strongly positive for S-100 and vimentin, but a positive reaction for vimentin was uncommon for neuroblastomas [2, 11–13, 15]. Oppedal *et al.* [12] reported the immunohistochemical features of human neuroblastomas and related normal tissues. In their report, neuroblastomas were always negative for vimentin. In addition, the positive reaction for both vimentin and S-100 protein has commonly been found in glioblastomas, undifferentiated astrocytomas, and malignant schwannoma [15]. Omi *et al.* [11] examined 5 cases of bovine nervous tissue tumors and showed that ganglioneuroblastoma and anaplastic ganglioglioma were strongly positive for vimentin and S-100, whereas a peripheral neuroblastoma was negative for both. However, Winkle *et al.* [18] recently described the immunohistochemical natures of central PNET in dogs and cats and demonstrated that all seven PNETs were positive for vimentin as those in human cases. Thus, the positive reaction for vimentin may be common feature for the PNETs. From these previous data together with our histopathological and immunohistochemical findings, we considered that the tumor in Case 1 should be diagnosed as

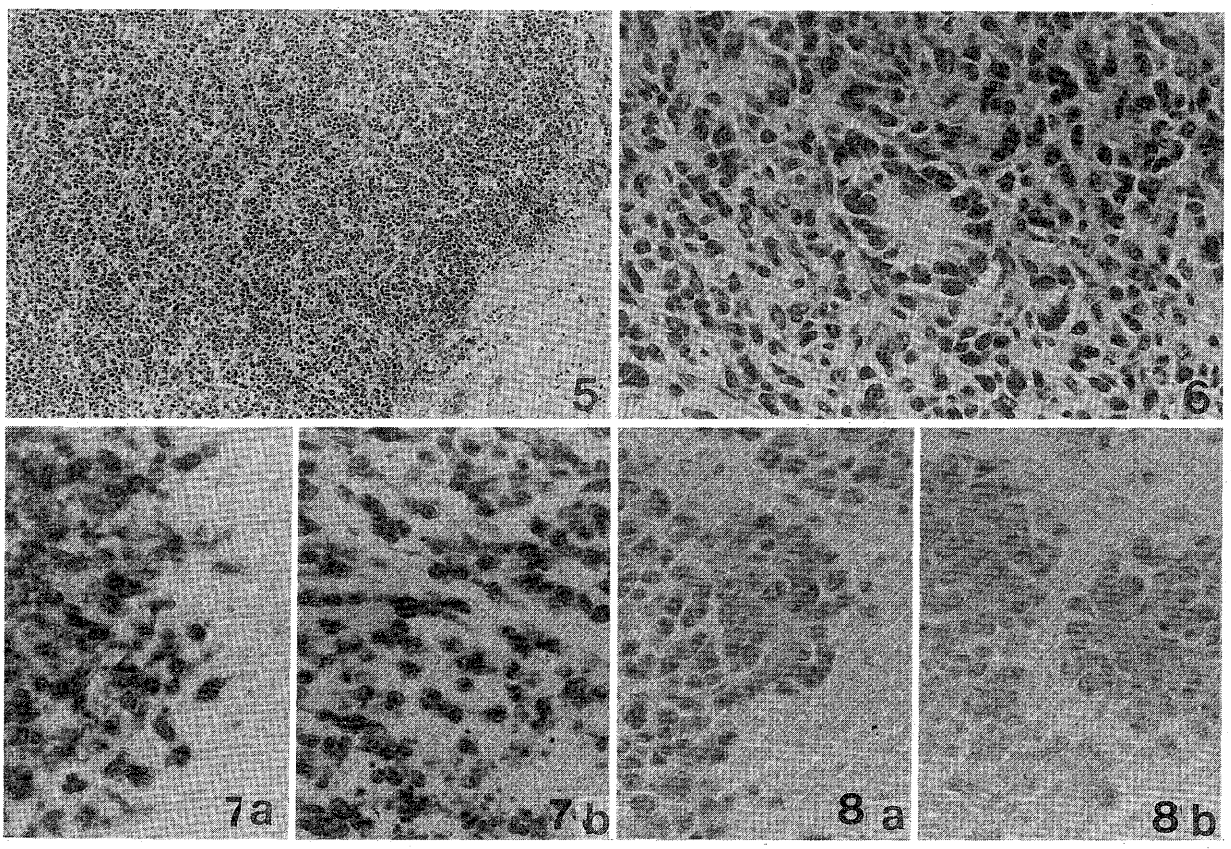

Fig. 5. Solid proliferation of round to cuboidal cells with clear cytoplasm and hypochromatic round nuclei. Case 2. HE. × 25.
Fig. 6. Homer & Wright rosette formation of the tumor cells. Case 2. HE. × 180.
Fig. 7. Immunoreactivity for vimentin (a) and NF (b) of tumor in Case 1. The cytoplasm of the tumor cells shows intense positive reaction for both vimentin (a) and NF (b). ABC method. × 200.
Fig. 8. Immunoreactivity for NF (a) and NSE(b) of tumor in Case 2. The cytoplasm of large tumor cells and stromal area within the rosettes show moderate to intense immunoreactivity for NF (a) and NSE (b). ABC method. × 200.

PNETs with a potential of neuronal differentiation. In contrast, the neoplastic cells in Case 2 showed intense immunoreactivity for both NSE and NF. Since many human and animal neuroblastomas have shown immunoreactivity for low or intermediate molecular mass NF and some have been positive for S-100 and NSE [10-12, 15, 19], the immunohistochemical natures of Case 2 are almost in conformity with those in neuroblastomas. In Case 2 small spindle-shaped cells positive for both S-100 and vimentin in the periphery of rosettes might be non-neoplastic stromal Schwann cells as described in human peripheral neuroblastomas [15].

In conclusion, the present paper describes the morphological and immunohistochemical features of bovine peripheral neuroblastoma and PNET, and suggests a combination of immunostaining for NF, vimentin, S-100, and NSE would be useful to know the degree of differentiation of the tumors with potential to differentiate for neuronal cells.

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寄生虫病学：

本邦のイヌにおける抗*Neospora caninum*(NC)抗体保有率を調査した．ネオスポラ症発症および抗NC抗体保有牛飼養農家で飼育されているイヌ48頭中15頭(31.3％)が抗体を保有していた．一方，都会で飼育されていたイヌ198頭中14頭(7.1％)が抗NC抗体を保有していた．抗体検査2カ月前にネオスポラ症が発生したブリーダー宅で飼育されていた7カ月齢以上の成犬17頭すべてが抗NC抗体を保有していた．1年半後に同ブリーダーにて再検査を行ったところ，抗体価に大きな変動はなかった．イヌのブリーダーと酪農家で飼育されていたイヌでNC抗体陽性率が著しく高かったことは，NCがイヌの間で水平伝播し，かつイヌとウシの間で水平伝播されている可能性を示唆するものであった．

病　理　学：

マレック病(MD)ウイルス1型実験感染SPF鶏から経時的に採材された羽髄病変(FPL)について細胞診と組織学的検索を行い両者を比較した．MDの内臓腫瘍または神経病変を持つ実験感染鶏では，感染初期に非化膿性炎症性反応がみられ後期にはリンパ芽球性腫瘍性病変がFPLの細胞診により認められた．一方，実験感染鶏のうちMDの内臓病変または神経病変いずれも生じていない個体については，実験期間を通して非化膿性の炎症性FPLが細胞診で観察された．同一鶏からの採材によるFPLの組織所見は細胞診と一致していた．これらの結果から，FPLの細胞診はMDの診断並びに予測をするための有効な手段であることが示された．

イヌ，8歳，雌の頭頂部皮膚ならびに歯肉に，多数のマクロファージと少数の好中球の浸潤からなる灰白色の肉芽腫性丘疹が認められた．浸潤マクロファージは腫大し，多数の卵円形の酵母様真菌を含んでいた．酵母様真菌は直径2－5 μm，周囲にハローを持ち，PAS染色，グロコット染色に陽性を示し，免疫組織化学的に抗ヒストプラズマ(yeast)抗体に陽性を示した．本例は日本におけるイヌヒストプラズマ感染症の初発例である．

黒毛和牛に認められた末梢性神経芽腫と未分化神経外胚葉腫瘍を病理学的に比較検索した．症例1は1歳の雄牛で，腫瘍組織が右側頭蓋腔と副鼻腔を置換し，右側下顎部には直径約18 cmの脆弱腫瘤の形成を認めた．症例2は7歳の雌牛で，剖検時に直径約15 cmの腫瘤が下顎部に認められた．病理組織学的に症例1では腫瘍細胞のPseudopalisade配列を伴う著明な壊死巣形成がみられ，一方，症例2ではHomer & Wright型のロゼット形成が顕著であった．免疫組織化学的には，症例1の腫瘍細胞はVimentin，S-100，およびニューロフィラメントに陽性を示し，症例2の腫瘍細胞はニューロフィラメントと神経特異エノラーゼに陽性で，Vimentin，S-100には陰性を示した．このような2症例間における相違は腫瘍細胞の分化度の差異を反映しているのではない

かと思われた.

生　理　学：

ホルスタイン雌牛から得た卵胞液中のカルシウム(Ca)とマグネシウム(Mg)の定量および両者のトロンビン形成における役割について血液成分と比較検討した．総Ca量は卵胞の発育に伴い増加したが，総Mg量は逆に卵胞液の貯留当初の胞状卵胞への発育初期に著しく高く，卵胞の発育に伴い漸次減少した．*in vitro*で卵胞液にMgを追加したところ，卵胞の直径が2 mm以下の卵胞から採取した卵胞液のプロトロンビン時間(PT)は短縮したが，3 mm以上の直径の卵胞から採取した卵胞液では影響が認められなかった．しかし，いずれの発育時期の卵胞液においても，ラッセル蛇毒によって活性化した第X因子活性に対しては　Mgの追加は作用の抑制を示した．以上の結果から，Ca濃度の低い卵胞腔形成初期にMgはトロンビン形成を緩やかに促進していることが明らかとなった．

公衆衛生学：

繁殖障害の乳牛における*Coxiella burnetii*感染状況を207例の血清および生乳を用いた間接蛍光(IF)抗体法，ネスティッドポリメラーゼ連鎖反応(PCR)および分離により検討した．IF抗体は，*C. burnetii*のⅠ相菌に対し122例(58.9％)が，Ⅱ相菌に対し125例(60.4％)が陽性を示した．PCRでは血清8例(3.9％)から，また生乳の51例(24.6％)から遺伝子が検出された．*C. burnetii*は，生乳の51例(24.6％)から分離された．以上の結果は，牛のコクシエラ症の診断にIFとPCRが有用であること，また，繁殖障害を伴う乳牛が動物およびヒトの重要な感染源の一つになる可能性を示唆している．

タイ国の人及び各種食品等から検出されたサルモネラの分布を明らかにする目的で，1993年から1996年にタイ国の人，鶏肉，惣菜およびエビから分離されたサルモネラ合計27,497株について血清型別を行った．人由来株は72血清型に，非人由来株は82血清型に型別された．得られた成績に基づいて，それぞれの食品がサルモネラ症を媒介する可能性について考察を加えた．

臨床繁殖学：

着床遅延時にDNA合成を含む胚の代謝が抑制されていることはマウスを含む多くの種で報告されているが，その機序は明らかにされていない．本試験は，マウスの着床遅延時に特異的に出現する子宮由来蛋白(DIAP170K)の胚のDNA合成抑制効果について，[^{3}H]thymidineの取り込みを指標として検討した．マウスの着床遅延は，妊娠3日目に卵巣を摘出し，プロジェステロン投与により誘起した．DIAP170Kは，50 μg/mlで着床