寄生虫病学

日本における Babesia caballi および Babesia equi 感染馬の血清学的疫学調査——管井安美, 永井章子, 宇学南, 五十嵐郁男, 震尾次彦, 池村, 小山田隆, 鈴木直義, 藤崎幸枝, 帯広畜産大学原虫病研究センター, 北里大学獣医学教室, 農業技術研究機構動物衛生研究所)

1971-1973年に全国的に集められた馬血清2,019頭について、Babesia caballi と Babesia equiに対する抗体調査を行った。組換え抗原を用いたELISAでは、B. caballi抗体陽性馬が5.4％（109/2,019）、B. equi抗体陽性馬が2.2％（44/2,019）であった。さらに、ELISA陽性馬血清を虫体抗原を用いたウエスタンプロット法により検査を行った所、109頭中30頭がB. caballi抗体陽性を示し、44頭中2頭がB. equi抗体陽性を示した。また、今回の血清では両種に対して陽性を示したものはなかった。以上の結果、日本の馬についてB. caballi と B. equiの再調査が必要と考えられた。

フタトゲチマダニの吸血に対するスカンベンジャーレセプター・ノックアウト(SRKO)マウスの免疫応答と抵抗性獲得——鈴木本智, Bhagat, N. A., 鈴木宏志, 南村, 小林, 小林宏志, 藤崎幸枝, 帯広畜産大学原虫病研究センター, 北里大学獣医学教室, 農業技術研究機構動物衛生研究所)

フタトゲチマダニ導入株とはマクロファージ・スカンベンジャー・レセプターA・ノックアウト(SRKO)マウスに3回連続して吸血させたところ、血清IFNγ濃度、マダニ抽出液抗原に対する抗体値、脱血体重減少を指標とした抵抗性獲得のいずれも、対照マウス(SR +/−)より有意に低いことが示された。この結果、マダニの吸血に対する免疫応答発現と抵抗性獲得において、マクロファージ・スカンベンジャー・レセプターAが重要な役割を果たしていることを示すものと考えられた。

病 理 学

犬における嗅神経上皮腫：免疫組織化学的並びに電顕的検索(短報)——原一弥, 岸田薰, 田村雅男, 森田剛仁, 速見信子, 鳥取大学農学部家畜病理学教室, 北里大学病院医学研究科比較病理学教室)

嗅神経上皮腫について電顕的並びに免疫組織化学的検索を実施した。10歳の雄犬の鼻腔内に腫瘍が認められ、症例は鼻出血、鼻汁排出および上頸部腫脹を示した。腫瘍組織は、立方形から円柱状細胞による管状構造および線維血管基質に囲まれた集族した小細胞により構成されていた。有絲分裂像が頻繁に認められた。腫瘍細胞は、免疫組織化学的に、サイトケラチン切、ニューロファイリン、シナプトフィジン、カルノシシンなどに陽性像を示した。電顕検索により、腫瘍細胞間で細胞間接着装置が認められ、フィブリンを含む分泌顆粒が認められなかった。これらの所見から、本腫瘍は、神経系並びに上皮性の特徴を有する腫瘍細胞から構成されること、および嗅上皮由来の腫瘍であることが示唆された。

薬 理 学

脱水牛への等張および低張醗酵乳酸加リン液の静脈内投与が循環血流量、中心静脈圧および血清電解質に及ぼす影響——原一弥, 藤村純子, 阿部, 白木由, 岩崎成規, 金山喜一, 浅野隆司, 日本大学生物資源科学部獣医学科, 日本全薬工業(株)中央研究所)

一日維持輸液量(30 ml/kg)の等張乳酸加リン液を脱水牛に静脈内投与し、中心静脈圧(CVP)および酸塩基平衡に及ぼす影響を検討した。中程度脱水(7.0%)は9頭のホルスタイン種未経産牛に対し、48時間の摂取および飲水を制限して作った。供試牛は無作為にILG(乳酸加リン液±5.0%ブドウ糖), HLG(1/1乳酸加リン液±
Seroepidemiologic Studies on Babesia caballi and Babesia equi Infections in Japan

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ABSTRACT. Antibodies to Babesia caballi and Babesia equi were examined on a total of 2,019 horse serum samples that had been collected in 1971-1973 by the National Institute of Animal Health by enzyme-linked immunosorbent assay (ELISA) using recombinant proteins and by Western-blot analysis. Based on the criterion for positivity by ELISA, 5.4% (109/2,019) and 2.2% (44/2,019) had antibodies against B. caballi and B. equi, respectively. The ELISA-positive sera were further examined by Western blot; 30/109 for B. caballi and 24/44 for B. equi were positive for native B. caballi or B. equi, but none of them was seropositive for both infections. Based on the results of this study, further investigations should be required to survey horses that have arrived in Japan relatively recently and tick vectors of equine Babesia using ELISA with some recombinant protein, a parasite detection method in an in vitro culture of equine Babesia, and PCR testing.

KEY WORDS: Babesia caballi, Babesia equi, ELISA, Japan, Western blot.


MATERIALS AND METHODS

Serum samples: Serum samples of 2,019 horses were collected at random from every prefecture in Japan from 1971 to 1973 by the National Institute of Animal Health for a survey of equine infectious anemia and were kept at -20°C until use.

ELISA: The ELISA was performed as described [10, 12, 23]. Briefly, B. caballi 48-kDa merozoite rhoptry protein (GST-BC48) expressed by a pGEX4T expression vector (Amersham Pharmacia Biotech, England) in Escherichia coli as glutathione S-transferase fusion protein and a recombinant baculovirus expressing B. equi merozoite antigen-1 (EMA-1), which were secreted into the supernatant of insect cell cultures, were used for the ELISA for B. caballi- and B. equi-infection, respectively [10, 12, 23]. Ninety-six-well microtiteration plates (Nunc-Immuno Plate; Nunc, Denmark) were coated with 50 μl of purified GST-BC48 (0.1 μg/ml) or the secreted EMA-1 diluted in a 0.05 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4°C. To reduce non-specific binding, the plates were blocked with PBS containing 3% skim milk (PBS-SM) for 1 hr at 37°C. The blocking agent was removed, and individual horse serum diluted at 1: 80 in PBS-SM was added to each well and then incubated for 1 hr at 37°C. After washing six times with PBS containing 0.05% Tween-20, 50 μl of peroxidase-conjugated goat anti-horse IgG antibody (Cappel, N.C.) diluted at 1:4,000 in PBS-SM was added to each well and incubated for 1 hr at 37°C. The plates were washed as described above, and then 100 μl of substrate solution (0.1 M citric acid, 0.2 M sodium phosphate, 0.003% H2O2, and 0.3 mg/ml 2′,2′-Azide-bis[3-ethylbenzthiazoline-6-sulfonic acid]) was added to each well. Absorbance at 415 nm was read after 1 hr incubation at room temperature using an ELISA reader (Corona micro-
plate reader MTP-120; Corona, Japan). The optical densities (OD) over 0.2 were interpreted as positive [10, 12, 23].

**Western blot:** The positive sera detected by ELISA were further analyzed by Western blot. Western blot was performed on an antigen prepared from B. caballi- and B. equi-infected erythrocytes obtained from microaerophilous stationary-phase cultures as described [11]. Horse serum samples diluted at 1:80 in PBS-SM and the peroxidase-conjugated goat anti-horse IgG (Cappel) diluted at 1:1,000 were used as first and second antibodies in Western blot, respectively. After being washed three times with PBS for 5 min, the samples were incubated with enhanced chemiluminescence (ECL) detection reagents (Amersham Pharmacia Biotech) for 1 min. In the case of ECL detection, the membrane was exposed to a film.

**RESULTS AND DISCUSSION**

When GST-BC48 and EMA-1 recombinant proteins were used in the ELISA, we detected B. caballi- and B. equi-specific in sera from 2,019 horses in Japan. Based on the criterion for positivity described above, 5.4% (109/2,019) and 2.2% (44/2,019) had antibodies against B. caballi and B. equi, respectively (Fig. 1a, b). These positive sera were further examined by Western blot using native antigens; 30/109 for B. caballi and 2/44 for B. equi were positive for native B. caballi or B. equi proteins, respectively, but none of them was seropositive for both infections. Western-blot analysis showed that ELISA-positive reacted with specific B. caballi proteins (major bands at 27, 29, 48, and 50 kDa; minor bands at 70 and 112 kDa) and with B. equi proteins (bands at 34 and 43 kDa) (Fig. 2).

Currently, regulatory control of equine babesiosis in the U.S.A., Brazil, Canada, Japan, and Australia, relies on serological tests [7]. Although CFT is the official test used by the USDA and Japan, it has been shown to yield both false-positive and false-negative results for B. caballi [5, 21, 22]. Horse sera in Japan were tested by IFAT, and some pseudo (suspicious)-positive sera of B. caballi and B. equi were detected [21]. However, some pseudo (suspicous)-positive sera of B. caballi and B. equi were not precisely ascertained to be equine Babesia infections. At present, ELISA using recombinant protein has the highest sensitivity for equine Babesia infections [10, 12, 14, 15, 20, 23]. In the present study, ELISA using recombinant antigens detected serologically positive cases, and specific bands were also observed in some ELISA positive ones by Western-blot analyses. Western-blot analysis has been suggested as a confirmatory test that provides a species-specific diagnosis [3]. Moreover, B. equi parasites were detected by an in vitro culture system from a horse with a Western-blot positive result [8].

The ELISA using GST-BC48 of B. caballi and EMA-1 of B. equi were considered that might enable detection of antibody to the protein up to a year or more after infection [11, 15]. Moreover, specific B. caballi or B. equi proteins with similar molecular weights as those reported by others [1, 2, 4, 9, 16] were detected in the present study. For B. caballi, Böse et al. [2, 3] analyzed proteins of B. caballi (USDA

![Fig. 1. a, b. Distribution of ELISA results using recombinant protein by OD value. A total of 2,019 horse serum samples were tested in ELISA with GST-BC48 (a) and EMA-1 (b).](image-url)
strain)-infected erythrocytes by Western blot with sera from horses that had been experimentally and naturally infected with *B. caballi*. Dominant proteins of 48, 50, 70, 112, and 141 kDa were recognized by European and Brazilian horse sera tested. In the present study, not only similar proteins of 48, 50, 70, and 112 kDa but also newly identified proteins of 27 and 29 kDa were recognized. For *B. equi*, a monoclonal antibody (mAb)-based competitive ELISA has been developed by Bruning et al. and Knowles et al. [4, 16, 17]. A mAb reacted with a 34-kDa surface protein of *B. equi* that had also been detected in immune sera of horses infected with different strains of the parasite. In the present study, the 43-kDa protein of *B. equi* was detected by Western blot. These results suggest the possibility that *Babesia*-infected horses might have invaded Japan from foreign endemic regions as a result of long-standing importation or that native equine *Babesia* has existed as an attenuated strain in Japan.

In conclusion, the present study suggests the existence of equine babesiosis in Japan by the detection of antibodies against *B. caballi* and *B. equi* in 1971–1973. The vectors for *B. caballi* and *B. equi*, such as *D. reticulatus* and *R. sanguineus*, have been reported in Japan [24]. *D. reticulatus* was confirmed in Japan in 1974, but the actual distribution has not been accurately determined [13]. Autochthonous infections have not been observed in Japan, although the potential vector, *H. longicornis*, also occurs in this country [13]. Thus, it is necessary that the actual conditions of the equine *Babesia* will be investigated in Japan. Concretely, further studies should be required to examine the horses that are definitely infected with equine *Babesia* and survey the tick vector of equine *Babesia* in Japan using ELISA with some recombinant protein of equine *Babesia*, a detection method in an *in vitro* culture of equine *Babesia*, and PCR testing.

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REFERENCES


