た。猫においては、1日量として0.5mg/kg以上のエナラブリルで同様なACE阻害効果が得られることが示された。

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——筏井宏実1),2)・永井章子1)・玄学南1)・五十嵐郁男1)・神尾次彦1),3)・辻尚利3)・小山田隆2)・鈴木直見1),3)・藤崎幸幸1)帯広畜産大学原虫病研究センター、2)北里大学獣医学部生虫学教室、3)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。組み換え抗原を用いた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討を行った所、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ではなかった。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。

フタゲミマダニの吸血に対するスカベンジャー・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——林本智志1)・Bhagat, N. A.2)・鈴木宏実1)・You, M.1)・神尾次彦3)・辻尚利3)・Claveria, F. G.1)・長澤秀行1)・豊田裕1)・藤崎幸幸1)帯広畜産大学原虫病研究センター、2)インド農業省病院局、3)動物衛生研究所、4)フィリップンデラサル大学生物学部）

フタゲミマダニによって集められたマウスに3回連続して吸血させたところ、血清IFN-y濃度、マダニ抽出抗原に対する抗体価、飽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+/+）よりも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：
犬における喉頭部上皮腫：免疫組織化学的並びに電顕的検索（短報）——原一弥1)・島田春則1)・森田剛仁1)・澤田清人1)・梅村孝司2)（1)鳥取大学農学部家畜病理学教室、2)北海道大学大学院獣医学研究科比較病理学教室）

喉頭部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、鼻汁排出および頭下腫脹を示した。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性血管基質に囲まれ密に集族した小細胞により構成されていた。有糸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、サイトカレンチンの他、ニューロフィラメント、シナプトフィビル、カルノシンなどに陽性像を示した。電顕検索により、腫瘍細胞間の細胞間接着装置が認められたが、有糸分裂像を持つ内分泌顆粒は認められなかった。これらの所見から、本腫瘍は、神経系並びに上皮性的特徴を有する組織細胞から構成されること、および喉頭部由来の腫瘍であることが示唆された。

薬 理 学：
脱水牛の不適および低張糖加乳酸加リンギル液の静脈内投与が循環血流量、中心静脈圧および血清浸透圧に及ぼす影響——鈴木由1)・奥村純子2)・阿部 泉2)・岩淵成弥2)・金山孝一1)（1)日本大学生物資源科学部獣医学科、2)日本全薬工業(株)中央研究所）

一日維持輸液量（30ml/kg）の低張乳酸加リンギル液を脱水牛に静脈内投与し、中心静脈圧（CVP）および酸価基準を変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断乳牛に対し、48時間の摂食および飲水を制限して作製した。供試牛は無作為にILG（乳酸加リンギル液＋5.0％ブドウ糖）、HLG（1/2乳酸加リンギル液＋
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 27/44 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

Equine babesiosis, also known as biliary fever, is an acute, subacute, or chronic tick-borne disease of Equidae caused by the hemoprotozoan parasites, Babesia caballi and Babesia equi. The disease, which is widely distributed in tropical and subtropical areas worldwide, causes significant economic loss to the horse industry. Babesiosis is generally characterized by fever, anemia, jaundice, and edema. In some cases, it causes the death of infected horses [4, 6, 18, 19]. Up to the present, no clinical equine babesiosis has been reported in Japan, and therefore Japan is still considered free from the disease. However, there has been a long-standing increase in the number of imported horses from foreign countries including endemic areas, and the existence of tick vectors, Dermacentor reticulatus and Rhipicephalus sanguineus, has also been reported in Japan [24]. In this recent survey, Dermacentor sp. seemed to have been exterminated in Japan; furthermore, Haemaphysalis longicornis has raised questions about the real vector of equine Babesia which was widely distributed in Japanese pastures [13]. These situations indicate that Japan is facing the risk of introducing infected or carrier horses. Although the complement fixation test (CFT) has been used as the official test to detect antibodies against equine Babesia parasites by the United States Department of Agriculture (USDA) and Japan, it has been shown to yield both false-positive and false-negative results for B. caballi [5, 6, 21, 22]. In this study, a serological survey of equine babesiosis in Japan was conducted on sera collected during 1971–1973 by enzyme-linked immunosorbent assay (ELISA) and Western blot using recombinant proteins and native proteins, respectively.

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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 (suspicious)-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 to 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](image-url) a. 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).
strains)-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]. An 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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