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A study on the epidemiology of caprine anaplasmosis in Korea
I. Electron microscopic characterization of the etiologic agent

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산양의 anaplasmosis 에 대한 역학적 연구
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초 록 : 우리나라에서 사육되고 있는 재래산양과 호주로부터 수입한 산양에서 빈혈, 식욕감퇴, 높은 발병율과 사망율을 나타내는 괴질이 발생하여, 이에 대한 원인을 밝히기 위하여 전자현미경적으로 충체를 관찰하였던 바, 적혈구내 단일막으로 위요되어 있는 기본소체와 이중막으로 둘러싸여 있는 봉입체가 관찰되었기에 이 질병의 병원체를 *Anaplasma ovis*로서 동정 보고하는 바 이다.

Key words : *Anaplasma ovis*, goats, electron microscopy

Introduction

Anaplasmosis is a worldwide disease of cattle and small ruminants causing losses through mortality, abortion and reduction in weight gain and milk production.[1] In the United States the annual economic loss due to bovine anaplasmosis alone was estimated at approximately half a billion dollars according to a USDA report in 1984.[2]

Efforts to develop an effective method for the control of this economically important disease have continued for more than 50 years. However, bovine anaplasmosis still remains one of the most severe constraints to livestock production in both tropical and subtropical regions of the world including the USA.[3] Cattle that have recovered from acute anaplasmosis remain persistently infected with a low level parasitemia and serve as a reservoir of the

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organism for infection of ticks or by mechanical transmission to other susceptible cattle.[4] *Anaplasma* species are obligate intraerythrocytic parasites in the order Rickettsiae which infect domestic and wild ruminants.[5-7]

In Korea, there have not been any reports so far on caprine anaplasmosis which causes a high mortality in these animals. In one farm alone, for example, 250 out of a total of 800 imported goats from a hitherto were sacrified with mysterious anemia during the Summer and Autumn of 1992. The disease was later diagnosed as anaplasmosis, based on light microscopic observations.[8]

Anaplasmosis caused by *Anaplasma marginale* or *Anaplasma centrale* occurs throughout the tropical and subtropical regions of the world, where it remains a major constraint to improved cattle production in many developing countries. It is important to note that based on our recent serologic data(unpublished data), Korea is free from bovine anaplasmosis caused by *Anaplasma marginale*, using the serologic criteria devoloped and recommended by the United State Department of Agriculture(USDA). All filter paper samples collected from korean cattle were found to be serologically negative by the USDA's complement fixation test(CFT) for bovine anaplasmosis. These data confirmed that bovine anaplasmosis did not exist in Korea. In this report, we discuss the observation of *Anaplasma* organisms associated with anemia and emaciation in indigenous and imported goats in Korea. Following a comprehensive investigation, the causative agent of the caprine anaplasmosis was shown to be *Analasma ovis* on the basis of light and ultrastructural characteristics.

Materials and Methods

Background and case histories : During the last 2 years, Korea has been importing a substantial number of goats from Australia. Recently farmers were concerned about a mysterious disease which they described as being of high morbidity and mortality. The animals became weak, anorexia and a significant number of them (250 among 800 imported goats) died during period of just one year. Among the native breeds the prevalence was somewhat high but comparable with the situation in exotic goats. The mortality among indigenous goats was not significantly higher than what was observed in imported goats. The introduction of the disease into Korea appeared to have developed recently, as no such reports were available previously. There was no parallel clinical problem in cattle grazing in the same environment as the affected goats.

Light microscopic appearance : The acridine orange stain was used throughout this study. Briefly, whole blood samples were fixed in absolute methyl alcohol for at least five minutes at room temperature. Smears were then made on microscope slides and stained with 0.1% acridine orange. They were then examined under ultraviolet light for evidence of definitive fluorescence revealing clear intraerythrocytic inclusions.

Transmission electron microscopy (TEM) : Infected red whole blood from a naturally infected indigenous goat in Changsugun was injected intramuscularly into goats that were immunocompromised by splenectomy. Blood samples from the splenectomized goat were collected by veinpuncture in both heparinized and plain serum tubes for parasitological, electromicroscopic and serologic analysis as described previously by Baek *et al.*[9] For TEM, the blood samples were fixed in reagent-grade 1.5% and 5% glutaraldehyde in cacodylate buffer. The sections of erythrocytes were fixed with 1% osmium tetroxide, dehydrated with ethanol and propylene oxide and infiltrated and polymerized in Lx112 epoxy resin. Thin sections(60-80nm) were cut with a diamond knife(LKB) placed on copper grids and stained with uracil and lead acetate. The sections were then examined using a JEOL 110cx electron ultramicroscope (Carl Zeiss, 10-CX).

Results

The splenectomized goat which was inoculated with whole blood from a natural case of anaplasmosis in an indigenous goat developed a disease with the

typical characteristic signs of anaplasmosis. *Anaplasma* organisms were observed in the peripheral blood 12 days post-inoculation. After staining with acridine orange, the *Anaplasma* bodies appeared as brilliant yellow-green, sharply defined round structures under ultraviolet light microscope.

The marginal bodies consisted of an outer matrix in which a variable number of initial bodies were observed. *Anaplasma* bodies were separated from the cytoplasm of the erythrocyte by a limiting membrane approximately 10μm in thickness. The electron density and thickness of this membrane were comparable to those of the plasma membrane of red blood cell. The inclusion bodies located intraerythrocytically were consistently observed (Fig 1). Most of the initial bodies were marginally located and predominantly single. They were surrounded by a limiting membrane. In cases of double bodies, these were enclosed in a double-layered membrane (Fig 2). Contents of the initial bodies were variable with some bodies being amorphous and empty. These ultrastructural characteristics of the agent in infected blood were consistent with *Anaplasma* ovis as the causative agent of caprine anaplasmosis in Korea. The induction of the disease in native goats confirmed the necessary Koch's postulates for the pathogenicity of the isolate.

Discussion

Anaplasmosis in goats and sheep is considered to be generally a mild disease presenting with fever, variable degrees of anemia and icterus with recovery in most cases leading to life long immunity associated with a carrier state.[10] Some breeds of goats may be more susceptible than others[11] and some strains of *Anaplasma ovis* may be more virulent for goats than for sheep.[12] In investigating the initial reports of the epidemic, it was hypothesized that the disease involved was a form of anaplasmosis although there was no previous evidence of the disease in Korea. However, we were prompted by the observation that the epidemic coincided with recent and on-going importation of goats from Australia, where anaplasmosis is endemic. Based on previous methodology,[8] the organism was verified as *Anaplasma* spp. The acridine orange staining method proved to be a reliable and simple diagnostic technique for this disease. Further, electron microscopic investigation confirmed very clearly that the agent was *Anaplasma ovis*. *Anaplasma ovis* is defined as a distinct species from *Anaplasma marginale* based on the intraerythrocytic location of the inclusion bodies and gost specificity.[13,14] The organism was predominantly single or bilobate intracellular inclusions (initial bodies) demarcated by a single-walled limiting membrane. Both initial bodies were surrounded by a double-layered membrane and the membrane-enclosed organisms were generally marginally located and were completely filled with 2 initial bodies.[15] The initial body was round or oval and was enclosed in a double membrane. The internal structure of this body consisted of one central and a few peripheral dense aggregates of fine granular material embedded in an electron-lucid substance. These morphologic features are all consistent with published descriptions of *Anaplasma ovis*[15,16] and distinct from *A. marginale*. In addition, serologic evidence (data not shown) confirmed that the etiologic agent was an *Anaplasma* spp using criteria described by Ssenyonga et al.[17] The goat industry is becoming increasingly important in Korea as reflected by the large number of goats imported, particularly from Australia, where the endemicity of anaplasmosis is high in various ruminants.

Thus the possibility that the Korean isolate is of Australian origin cannot be precluded. However, no previous studies had been carried out in Korea since they were not necessary until the reported epizootic. It is anticipated that the current data will stimulate research in this area.

An interesting issue is how the disease was transmitted. Korea has a limited number of competent biologic vectors for *Anaplasma* species and the only important ticks is *Haemophysalis longicornis*.[18] Accordingly, the disease could have been spread by either a biologic vector or by mechanical means.

The fact that no cases of bovine anaplasmosis were reported or even suspected, in cattle grazing together with goats is further indication that the agent is

Anaplasma ovis. However, further studies are underway to verify the resistance of splenectomized and intact cattle to this agent. *Anaplasma ovis* does not cause disease in intact or splenectomized cattle but spleen-intact goats often develop a severe clinical disease.[14,1] *Anaplasma* ovis is also an important sheep pathogen capable of inducing a subclinical disease in intact animals, but causing an acute infection in splenectomized sheep.[14] The white tailed deer, *Dama virginiana*, are also susceptible to *A ovis* infection.[16] Finally, the finding that the disease was predominantly afflicting young female goats deserves further studies utilizing larger numbers of animals surveyed. If this observation turns out to be true, then one must be concerned about the potential role of *Anaplasma ovis* in causing abortion and associated complications in sheep and goats in Korea. Finally, the deer industry is beginning to pick up in Korea and it has been shown that *Anaplasma ovis* is pathogenic for the white-tailed deer.[16] Further studies will include comparison of antigenicity among different isolates from various areas in Korea. The latter will include islands where only indigenous goats are kept and certain islands that have restricted themselves to imported goats. The *Anaplasma* isolate from the goat should also be differentiated for *Anaplasma centrale*[19] using a specific DNA probe[20] and antigenically.[21,22] *Anaplasma marginale* and *A ovis* overlap significantly in their prevalence in tropical and sub-tropical conditions indicating that they are transmitted by similar mechanisms.[23] There was no concurrent incidence of bovine anaplasmosis in cattle grazing together with the sick goats indicating the relative host predilection of the agent. These data are discussed in relation to the importance of the goat industry in Korea and the potential epidemiologic significance to other susceptible animals, especially deer. The possibility that the organism may have been imported into the country cannot be precluded. Possible modes of transmission are discussed. Eradication of this disease in the USA was not possible using the control measures which were effective against babesiosis, partly due to the inability to satisfactorily detect infected animals.[24] The proposed approach will enable us to delineate the epidemiology of the disease and provide a rational strategy for the control of this disease that threatens the goat, sheep and deer industry in Korea.

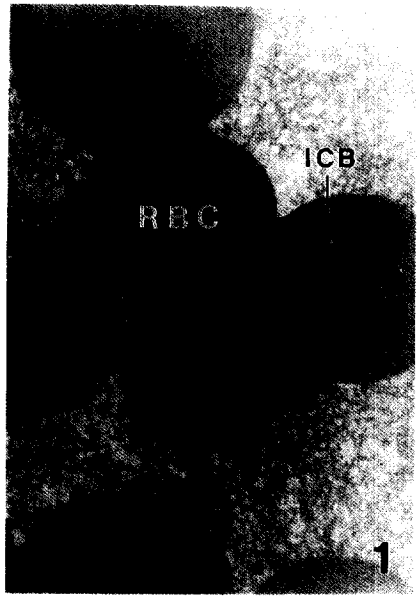
Summary

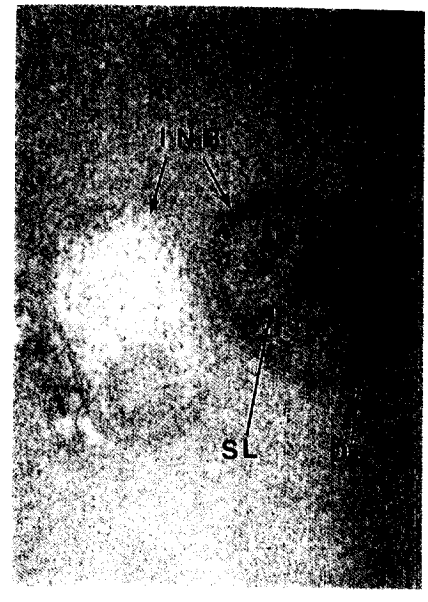
Evidence is presented for the isolation and characterization of *Anaplasma ovis* in both indigenous (Korean) and exotic goats imported from Australia. These studies were carried out in response to epidemic scenario whereby farmers reported noticing what was described as a mysterious disease characterized by anemia, anorexia, general malaise and a significant morbidity and mortality rate in both types of goat breeds. The syndrome consistent with caprine anaplasmosis was associated with an intraerythrocytic agent occuring in single initial bodies characteristically surrounded by a single-layered membrane whereas the marginal body was typically surrounded by a double-layered membrane. The identity of the etiologic agent was confirmed as *Anaplasma ovis* by light and ultrastructural microscopy.

Legends for figures

Fig 1.: A Single marginal body located in the cystoplasm of the red blood cell. X 3,000.
Remarks; RBC: red blood cell, ICB: inclusion body

Fig 2.: Two initial bodies demarcated by a double membrane. X 10,000.
Remarks; INB: initial body, SL: single layer membrane, DL: double layer membrane





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