Identification of *Eimeria brunetti* Using Glucose Phosphate Isomerase and Lactate Dehydrogenase

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Eimeria brunetti is one of the pathogenic species of chicken coccidia [2, 3, 4, 8]. However, not much has been known concerning its recent existence and distribution in Japan. Moreover, no reference strain of *E. brunetti* is available in Japan. Therefore, we surveyed the faecal samples of coccidia obtained from the field in order to establish the strain of *E. brunetti* and found two strains (No. 55 and No. 44) of *E. brunetti* from poultry farms. For investigating the characteristics of these two strains, experimental infection in 2-week-old specific-pathogen-free chickens which had been reared in our laboratories was performed by the methods of Long *et al.* [4]. Their characteristic profiles are summarized in Table 1. Briefly, oocysts were ovoidal shape and were larger than those of the *E. tenella* type.

The prepatent period was about 5 days (No. 55: 120 hr and No. 44: 124 hr). They were mainly observed in the lower small intestines and rectum. For genetical comparison of *E. brunetti* with other coccidia species, four reference strains of *E. tenella*, *E. acervulina*, *E. necatrix* and *E.*

maxima in Japan and one field isolate of *E. praecox* were used. The former four strains were kindly supplied by Dr. Tsunoda (National Institute of Animal Health, Ibaraki) and the latter was isolated from poultry farm as described previously [6]. The enzyme samples prepared from oocysts of strains Nos. 55 and 44 were subjected to starch-gel electrophoresis using glucose phosphate isomerase (GPI) and lactate dehydrogenase (LDH) according to the method introduced previously [6, 7]. Both enzyme spots of these two strains migrated faster than those five other species of chicken coccidia (Fig. 1), in agreement with the results of Shirley [9] and Shirley and Rollinson [10]. Thus, these two strains were confirmed to be *E. brunetti* on the basis of its experimental infection and electrophoretic patterns.

Enzyme electrophoresis is now recognized as an important characterization method [5, 10] and can be used to identify several coccidia species present in a mixture [1]. The primarily collected samples of faeces contained several species of oocysts. The chickens were orally inoculated with each of these two oocysts and reproduced oocysts

Table 1. Characteristics of two strains (No. 55 and No. 44) isolated from poultry farms

Oocyst morphology

Shape : Ovoid
Size : Length 24-29 μm (Mean : 26 μm)
Width 19-22 μm (Mean : 21 μm)
Color : None

Prepatent period

No. 55 : 120 hr
No. 44 : 124 hr

Parasite location

Small intestines			Rectum	Bursa of fabricius
Anterior	Middle	Posterior		
+	++	+++	+++	+

The symbols, +~+++, show the numbers of parasites observed in a section (+: rare, ++: a few, +++: many).

Fig. 1. Electrophoretic patterns of glucose phosphate isomerase (A) and lactate dehydrogenase (B). 1. *E. acervulina* 2. *E. praecox* 3. *E. maxima* 4. *E. brunetti* (No. 44) 5. *E. brunetti* (No. 55) 6. *E. necatrix* 7. *E. tenella*.

were collected from the faeces 100–170 hr after inoculation. Several passages in chickens were required to provide sufficient oocysts for electrophoresis [1]. The enzyme samples prepared from these sporulated oocysts were subjected to enzyme electrophoresis using GPI and LDH. The zymographs of GPI and LDH showed the electrophoretic patterns of several species of coccidia including *E. brunetti*. So the single oocyst was isolated from each of these two samples to establish the strain of *E. brunetti*. Our results show that enzyme electrophoresis is a useful method for determining the species present in faeces.

Two strains were isolated from poultry farms in Hokkaido (No. 44) and Kumamoto (No. 55), the northern and southern parts of Japan which suggests extensive spread of *E. brunetti* in Japan. Further surveys using enzyme electrophoresis are needed to clarify the distribution of chicken coccidia.

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要 約

GPI および LDH アイソザイムによる *Eimeria brunetti* の分離(短報)：中村貴史・川口陽資・妹背 醇(塩野義製薬油日ラボラトリーズ)——鶏コクシジウムの病原性種として重要な *E. brunetti* の我が国における分布および感染の実態は不明な点が多い。そこで今回、野外から *E. brunetti* の分離を試み、2株を同定した。複数の種が混在する野外サンプルの場合、オーシストの形態、プレパテントピリオドおよび腸管の感染部位等の性状に加え、GPI および LDH アイソザイムを指標とした酵素電気泳動法の併用が有用であった。