DIFFERENTIATION OF SPECIES OF EIMERIA FROM THE FOWL USING A COMPUTERIZED IMAGE-ANALYSIS SYSTEM

J. KUČERA and M. REŽNICKÝ

Research Institute of Feed Supplements and Veterinary Drugs, 254 49 Jílovo near Prague, Czechoslovakia

Abstract. Oocysts of Eimeria species from the fowl have been identified using a computerized image-analysis system (Leitz T.A.S. Plus Image-Analyser). The system enabled semiautomatically measurement of oocyst dimensions with subsequent species diagnosis based on graphic statistical evaluation of size and shape of measured parasites. E. mitis, E. acervulina, E. brunetti, E. maxima, E. tenella and complex of E. necatrix and E. praecox were distinguishable both in pure cultures and in mixtures. It was not possible to distinguish E. praecox from E. necatrix using this system.

Oocysts of most Eimeria species from the fowl are difficult to distinguish when present in mixed cultures because their morphology is very similar and they overlap considerably in size (Long and Reid 1982). In this paper we have tried to identify the species of Eimeria present in mixed cultures by measuring the average length and width of oocysts with subsequent statistical evaluation using a computerized image-analysis system.

MATERIALS AND METHODS

Parasites. Oocysts were recovered from faecal samples of infected chickens by salt flotation, sporulated in 2 % potassium bichromate (Davis 1973) and stored for up to six months in 2 % potassium bichromate at 4 °C prior to measurement. A list of strains used in the present study:

E. mitis

E. acervulina

E. praecox

E. tenella

E. brunetti

E. maxima
Cultures are designated with serial numbers corresponding to those in Fig. 6. Details presented include the number, locality and year of isolation of each culture. p. = passage; h p.i. = hour post infection of oocyst collection.

**Preparation of oocysts for microscopy**

A small drop of a suspension of oocysts was placed on a clean glass slide and covered with a cover glass. Excess fluid was absorbed on a piece of filter paper and the cover glass sealed with liquid paraffin to prevent the preparation from drying out.

**Oocyst measurement**

Oocysts were measured with a TV-based image analysis system comprising a Leitz Orthoplan microscope, Bosch TV-camera and Leitz T.A.S. Plus Image-Analyser connected to an Epson printer and Hewlett-Packard plotter. Parasites were observed under phase contrast illumination with an objective lens 40× at a total magnification of 500. Oocysts were measured from the black-and-white TV-image on the computer screen using a semiautomatic programme that had been written by one of the authors (M.R.). The operator selected the oocysts to be measured with a light pen (part of the analyser for manual control of the analysis). Length and width of each oocyst measured were designated by touching the screen with the light pen at end points of these dimensions. The oocyst was then automatically given a serial number and its dimensions were depicted, together with shape index (length/width) (Fig. 1). The data were stored on a computer diskette for further evaluation. Usually fifty oocysts were measured from each culture, but if the culture was suspected to contain more than three species, one hundred were measured. Nonsporulated and deformed oocysts and those lying obliquely to the plane of focus were not measured.

![Fig. 1. The screen of the Leitz T.A.S. Plus Image-Analyser during measurement of oocysts of E. acervulina. Oocysts that have been measured are marked by serial numbers and their dimensions depicted: (from the left) serial number, length and width (in μm) and shape index.](image)

**Statistical analysis**

1. **Oocyst size.** The difference between length of the smallest and largest oocysts in a given set was divided by the computer into eleven equal intervals and all oocysts measured were allocated to these intervals according to their length. The calculated distribution was plotted as a “length histogram” (Fig. 2).
Fig. 2. Histogram of the distribution of oocyst length of a mixture of *E. mitis*, *E. acervulina*, *E. necatrix*, *E. praecox*, *E. tenella*, *E. brunetti* and *E. maxima*. MI, AC, N, P, TE, BR, MA — median values of oocyst length based upon pure strains of each species (see Fig. 3).

Fig. 3. Correlation between oocyst length and shape index from the same culture as in Fig. 2. Crosses show median values of different poultry *Eimeria* species: MI — *E. mitis*, 14.3 μm (length), 1.09 (shape index); AC — *E. acervulina*, 17.5 μm, 1.24; N — *E. necatrix*, 20.4 μm, 1.19; P — *E. praecox*, 21.0 μm, 1.18; TE — *E. tenella*, 22.8 μm, 1.26; BR — *E. brunetti*, 27.2 μm, 1.24; MA — *E. maxima*, 32.1 μm, 1.33. (Calculated from data from Figs. 4 and 6).
2. Oocyst shape was evaluated by calculating a shape index which is a quotient of the length and width of each oocyst (Long and Reid 1982). The values of the shape index were correlated with the length of each individual oocyst and this correlation was graphically plotted as a “correlation diagram” (Fig. 3).

Species diagnosis. A protocol of measurement of a studied culture comprised printed dimensions and values of shape index of each oocyst and the above described “length histogram” and “correlation diagram”. The final diagnosis was done by comparing the protocol with “length histograms” and “correlation diagrams” of pure cultures of individual *Eimeria* species.

**RESULTS**

Mean values for oocyst length and shape index of thirty different cultures of six species of *Eimeria*, plus data obtained from the literature for four cultures of *E. necatrix*, are shown in Fig. 6. These data were used for calculating median values of oocyst length and shape index of each species (see Explanation of Fig. 3). These values are also depicted in “length histograms” and “correlation diagrams” of measured cultures for helping the species diagnosis (e.g., Figs. 2 and 3).

![Variation of different Eimeria species in oocyst length. Total variation (-----), arithmetic mean (o), and median value (I) were calculated from measurements of different cultures (see p. 107) and from other authors (data in E. necatrix — see Fig. 6). MI — E. mitis, AC — E. acervulina, N — E. necatrix, P — E. praecox, TE — E. tenella, BR — E. brunetti, MA — E. maxima. Histograms show the distribution of oocyst length for cultures K748 (MI), K698 (AC), K764 (P), K770 (TE), K754 (BR) and K753 (MA) (see the list).](image_url)

The “length histograms” and “correlation diagrams” of pure cultures of six *Eimeria* species are compared in Figs. 4 and 5. Species with small oocysts (*E. mitis* and *E. acervulina*) are distinguishable according to both the oocyst length and shape index, which is clearly visible in the “correlation diagrams” by the position of clusters of points showing dimensions of individual oocysts. *E. praecox* differs from *E. tenella* by smaller and more spherical oocysts. Even if many oocysts of these two species overlap in their dimensions, *E. praecox* is usually indicated by the frequent presence
of oocysts with shape index under 1.18. Among the species with large oocysts, *E. maxima* is distinguishable from *E. brunetti* according to oocyst size.

The method of differentiation of *Eimeria* species using the computerized image-

---

**Fig. 5.** Diagrams showing correlation between oocyst length and shape index for cultures K748 (*E. mitis*), K698 (*E. acervulina*), K764 (*E. praecox*), K770 (*E. tenella*), K754 (*E. brunetti*) and K753 (*E. maxima*). See list of strains and Fig. 3 for more details. Compare Fig. 4.
-analysis system has been used for morphological characterisation of cultures either of laboratory strains or those isolated from different poultry farms. An example of “length histogram” and “correlation diagram” of a mixture containing *E. mitis* (44 % of the total number of oocysts), *E. acervulina* (11 %), *E. tenella* (9 %), *E. brunetti* (17 %) and *E. maxima* (19 %) is shown in Figs. 2 and 3. Three species (*E. mitis, E. brunetti* and *E. maxima*) are easily recognizable both in the “length histogram” and “correlation diagram”, while the remaining two species, being less abundant, are depicted not very distinctly by a few points in the “correlation diagram” only.

Fig. 6. Correlation between mean shape index and mean length of oocysts of different cultures of poultry *Eimeria*. Cultures measured are numbered as in List (page 107). Intervals of reliability on 95 % level are depicted in cultures Nos. 1 and 30, only. They have similar range in other cultures. Data for *E. necatrix* are from Tyzzer et al. (1932 — No. 10), Beeker et al. (1955 — No. 11), Edgar (1955 — No. 12) and Davies (1956 — No. 13).

**DISCUSSION**

Fully automatic measurement of coccidian oocysts was possible using the image-analysis system. However, although the image-analyser was able to recognize oocysts examined under phase contrast illumination, the practical application of the automatic programme revealed several difficulties that complicated the measurements. For example, separation of individual oocysts present in clusters was not always satisfactory and the image-analyser often measured such clusters as one object. Furthermore, the system was not able to distinguish sporulated from unsporulated or deformed oocysts and very often measured oocysts lying obliquely in the focal plane so that the dimensions were inaccurate. Also small particles attached to the surface of oocysts often resulted in false measurements. The presence in one field of vision of oocysts with distinctive sizes (e.g. *E. maxima* and *E. mitis*) made it difficult to focus the oocysts correctly. These difficulties were overcome by measuring oocysts directly on the computer TV-screen. The method was quicker than measuring parasites with an ocular micrometer in a light microscope and the results could be directly evaluated by the computer.
There is considerable variation in the length of the oocysts of *Eimeria* species and this has led some authors to consider that “it is doubtful if species other than *E. maxima* and *E. mitis* can be differentiated by this character alone” (Long and Reid 1982). Fig. 4 shows that oocysts larger than ca 34 μm and smaller than ca 14 μm can be identified as one of these two species with almost 100% certainty. However, providing sufficient numbers of oocysts are present, other species can also be detected since they produce a distinctive peak in average oocyst length in the “length histogram”. This is true especially in mixed cultures of *E. mitis, E. acervulina, E. brunetti* and *E. maxima. E. necatrix, E. praecox* and *E. tenella* have oocysts very similar in size so that mixtures of these species are hardly distinguishable because they usually produce one common peak in the “length histogram”.

Variation in the shape index of individual oocysts is even greater than variation in the length and all species overlap considerably in this character (Fig. 5). The shape index can be useful for species diagnosis when evaluated in correlation with the length, because most *Eimeria* species are characterized by the typical form of the “correlation diagram” (Fig. 5). Such a correlation may help to distinguish between species whose oocysts have similar length but differ in shape, e.g., *E. mitis* and *E. acervulina*, or *E. praecox* and *E. tenella*. However, *E. necatrix* and *E. praecox*, whose oocysts are similar both in size and shape, are indistinguishable (Fig. 6).

In conclusion, the method of image-analysis described is a useful tool for differentiation between *E. mitis, E. acervulina, E. brunetti, E. maxima, E. tenella* and complex of *E. praecox* and *E. necatrix*. It is more objective and possesses several advantages compared with conventional methods for measuring oocysts. However, it is still limited by the fact that oocysts of some species are morphologically similar and overlap considerably in both the length and shape index. This may cause problems especially in evaluating mixtures of more species with considerably different proportions in numbers of oocysts. It is therefore recommended that results achieved by the present method should be verified using other diagnostic characters (Long and Reid 1982, Shirley 1986, Kučera 1991, etc.).

REFERENCES


EDGAR S. A. 1955: Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. J. Parasitol. 41: 214—216.


Received 7 December 1989
Accepted 1 April 1990

113