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# IDENTIFICATION OF SPECIES OF EMERIA OF TURKEYS USING REGRESSION ANALYSIS OF MORPHOMETRIC INDICATORS OF OCYCISTS

**Petro Liulin, Mykola Bogach, Oleg Getmanets**

***The aim.*** *Identify the species of oocysts of turkeys by morphometric parameters.*
***Material and methods****. Eimeria oocysts obtained from faeces of suspects and patients with spontaneous eimeriosis of turkeys of poultry farms of Kharkiv region served as material for the research. Methods used: parasitological, coprological, light microscopy, morphometry, mathematical and statistical, correlation and regression analysis, ANOVA variation statistics.*
***Results.*** *Morphometrically (n=255) samples of turkey eimeria oocysts were studied and identified to the species: E. gallopavonis (n=50), E. meleagrimitis (n=50), E. adenoids (n=51), E. meleagridis n=53), E. innocua (n=51) according to identification indicators (X1 - length of the oocyst in μm; X2 - width of the oocyst in μm; X3 - area of the oocyst in μm; X4 - eccentricity of the model ellipse; X5 - ratio of the width of the oocyst to its length; X6 - largest curvature and X7 - smallest curvature in its model ellipse poles on the major and minor axes, respectively, in μm, X8 - presence - 1 or absence - 0 polar granules) which are mathematical expressions of morphometric dependences of the structure of eocystic oocysts which are confirmed by the results of regression and correlation analysis. The dependence of the Y oocyte species on seven characteristics has been proved.*
***Conclusions.*** *The morphological features that are mathematical expressions of morphometric dependences of the structure and identification of the species of turkey oocysts are determined with high accuracy. The relative error in determining the type of turkey eimerias does not exceed 2 %*
***Keywords****: identification, eimeria oocysts, turkeys, morphometry, regression analysis, correlation*

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## 1. Introduction

On the way to the industrialization of poultry farming, especially the breeding of turkeys Eimeriosis is a serious problem, could cause significant economic losses even in the subclinical course [1, 2]. In turkeys, eimeriosis is caused by seven types of pathogens Eimeria meleagridis (Tyzzer.1929), Eimeria dispersa (Tyzzer, 1929), Eimeria meleagrimitis (Tyzzer, 1929) [3–6], Eimeria gallopavonis (Hawkins, 1952), Eimeria adenoides (Moore and Brown, 1952), Eimtria innocua (Moore and Brown, 1952), Eimeria subrotunda (Moore, Brown, Carter, 1954) [7–11], belonging to the subkingdom of Protozoa, belonging to the subkingdom Protozoa, supertype Alveolata (Cavalier-Smith, 1991), infratype Apicomplexa (Levin, 1970), class Sporozoa (Leuckart, 1879), subclass Coccidia (Leuckart, 1879), series Eucoccidiida, suborder Eimeriina, family Eimeriidae, subfamily Eimeriinae, genus Eimeria [1, 11–14]. The causative agents of turkey eimeriosis were first discovered and described by T. Smith (1895) under the names Coccidium oviforme and Coccidium tenellum, which were similar and previously described in rabbits and chickens [9]. R. Hadlei (1911) isolated eimeria oocysts from turkey and described them as a species of Eimeria avium. W.T. Jhonson reported on the specificity of Eimer species in turkeys (1923) [7, 8]. reported the presence of independently existing species of eimeria in turkeys, and E. Tyzzer described in detail the causative agents of eimeriosis of turkeys such as E. meleagridis (E. Tyzzer, 1929), E. meleagridis (E. Tyzzer, 1929) and E. disperga (Tyzer, 1929) [8–10].

Up to now, the identification of pathogens of eimeriosis of turkeys is carried out according to their original descriptions and data determinants [15], consid-

ering the localization in the intestine. oocyst morphology, biology, longitude of prepatent and patent periods, presence of cross-immunity. However, some of these parameters may coincide or be defined insufficiently, which complicates their identification.

Analyzing the data of the literature on the morphology and biology of turkey eimeria [13] it should be noted that during the research there were contradictory and ambiguous results, which did not correspond to the original descriptions of pathogens, which indicated imperfection and difficulty in determining species [5, 16] and required comparison of the obtained results with descriptive materials [3, 4, 8, 15] and the location of pathogens in the body of turkeys. The aim of our work is to establish the type of oocysts of eimeria of turkeys by morphometric parameters.

There are also pathomorphological methods for diagnosing eimeriosis, which consider the peculiarities of the endogenous cycle of pathogens, their location in the intestinal tract and the presence of characteristic pathological and histopathological changes [5, 16], taking into account the morphology of oocysts, longitude (terms) of prepatent and patent development. Significant progress in the identification of causative agents of turkey eimeriosis has been the development of real-time PCR methods [17, 18] and mitochondrial genome [18, 19]. However, to this day, the identification of oocysts by morphological characteristics remains relevant. There is a method of identifying Eymeric oocysts, which consists in measuring the smallest and largest radii of curvature of the poles of the oocyst contour, complex calculations of the species indicator by the value of which identify the type of Eymeris [20], but to measure the curvature of oocysts is extremely difficult.

Therefore, further research is needed to improve the identification of pathogens of eimeriosis by morphological indicators.

**2. Materials and methods**

The research was conducted in the scientific laboratories of the Department of Pharmacology and Parasitology State Biotechnological University, laboratory of Epizootology and Parasitology Odessa Research Center, National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" National Academy of Agrarian Sciences of Ukraine, laboratory of Department of Artificial Intelligence, Faculty of Computer Science, Kharkiv National V. N. Karazin University

Material for the research (faeces) was obtained from suspected and clinically ill Eimeriosis turkeys of specialized poultry farms of Kharkiv region.

The material for the study was Eimeria srp oocysts isolated by flotation from the faeces of turkeys with suspected or diagnosed eimeriosis [21].

Morphometry of Eimeria oocysts (n=255) (measurement of oocyst length and width) was performed on an Axioskop–40 microscope magnification (×400) using an eyepiece micrometer, pre-determining the value of its scale (division price) of the object with a micrometer. Then the photos of oocysts were processed using the Adobe Photoshop CS6 13.1.2 program. The linear size of the oocyst was determined accurate to about 0.05 μm.

It has been was defined eight explanatory factors to define species of pathogen:

$X_1$ – the length of the oocyst in μm;

$X_2$ – the width of oocysts in μm;

$X_3$ – area of oocysts in $\mu m^2$;

$X_4$ – eccentricity of the model ellipse, the major axis of which is the length of the oocyst, and the minor one - the width of the oocyst, eccentricity is determined by the formula $X_4 = \sqrt{1-\frac{X_2^2}{X_1^2}}$;

$X_5$ – the ratio of the width of the oocyst to its length $\left( X_5 = \frac{X_2}{X_1} \right)$;

$X_6$ – the largest curvature of the model ellipse at its pole on the major axis in $\mu m^{-1}$, which is determined by the formula: $X_6 = \frac{2X_1}{X_2^2}$;

$X_7$ – the smallest curvature of the model ellipse at its pole on the minor axis in $\mu m^{-1}$, which is determined by the formula: $X_6 = \frac{2X_1}{X_2^2}$;

$X_8$ – dichotomous feature which is defined as the presence (value 1) or absence (value 0) of the polar granule.

It was introduced dichotomous (boolean) variable Y to specify different species of turkeys' oocysts of Eimeria:

Y=1 (for the species E. innocua);
Y=2 (for the species E. meleagrimitis);
Y=3 (for the species E. meleagridis);
Y=4 (for the species E. gallopavonis);
Y=5 (for the species E. adenoides).

Ranking was performed by the values of length and area of oocysts from the smallest to the largest.

To process the results of research were used the methods of correlation and regression analysis, as well as analysis of variance (ANOVA) [22].

The multiple correlation analysis methods were used to define the species of oocysts of Eimeria Y by the parameters $X_1 – X_2$ using "Correlation" tool from "Data analysis" package in MS Excel program [23].

**3. Results**

According to the measured values of width and length of oocysts of Eimeria (Table 1) it was calculated the following parameters:

area of oocysts – X3;
eccentricity – X4;
the ratio of the oocyst's width to its length – X5;
the largest and smallest curvature of the ellipse – X6, X7;
such morphological parameters as the presence (1) or absence (0) of polar granules – X8.

The following correlation matrix (Table 2) was build using the data from Table 1.

Table 1

The value of explanatory factors, M±m

| Species of Eimeria | X1, μm | X2, μm | X3, $\mu m^2$ | X4 | X5 | X6, $\mu m^{-1}$ | X7, $\mu m^{-1}$ | X8 |
|---|---|---|---|---|---|---|---|---|
| E. gallopavonis (n=50 ) | 24.37±0.09 | 16.11±0.07 | 308.5±2.1 | 0.750±0.002 | 0.661±0.002 | 0.188±0.001 | 0.054±0.000 | 0 |
| E. meleagrimitis (n=50) | 19.69±0.03 | 15.55±0.05 | 240.6±1.1 | 0.613±0.002 | 0.790±0.002 | 0.163±0.001 | 0.080±0.000 | 0 |
| E. adenoides (n=51) | 24.72±0.1 | 16.77±0.08 | 325.8±2.9 | 0.735±0.002 | 0.678±0.002 | 0.176±0.001 | 0.054±0.000 | 1 |
| E. Meleagridis (n=53) | 21.98±0.1 | 17.42±0.1 | 301.1±2.9 | 0.609±0.003 | 0.793±0.002 | 0.145±0.001 | 0.072±0.000 | 0 |
| E. innocua (n=51) | 16.10±0.01 | 15.33±0.04 | 193.9±0.5 | 0.301±0.006 | 0.952±0.002 | 0.137±0.001 | 0.118±0.000 | 0 |

Table 2

Correlation matrix for explanatory and explained parameters

| *Features* | *X1* | *X2* | *X3* | *X4* | *X5* | *X6* | *X7* | *X8* | *Y* |
|---|---|---|---|---|---|---|---|---|---|
| *X1* | 1 | | | | | | | | |
| *X2* | 0.6032 | 1 | | | | | | | |
| *X3* | 0.9675 | 0.7839 | 1 | | | | | | |
| *X4* | 0.9299 | 0.3883 | 0.8416 | 1 | | | | | |
| *X5* | –0.9453 | –0.3319 | –0.8374 | –0.9822 | 1 | | | | |
| *X6* | 0.7107 | –0.1288 | 0.5104 | 0.8264 | –0.8877 | 1 | | | |
| *X7* | –0.9764 | –0.5049 | –0.9149 | –0.9855 | 0.9791 | –0.7780 | 1 | | |
| *X8* | 0.4973 | 0.7681 | 0.6283 | 0.3509 | –0.3013 | –0.0611 | –0.4321 | 1 | |
| *Y* | 0.9546 | 0.5217 | 0.9083 | 0.8704 | –0.9086 | 0.7157 | –0.9187 | 0.5734 | 1 |

It was indicated a strong correlation between the species of oocyst Y and its length $X_1$ (correlation coefficient 0.9546), the total area $X_3$ (0.9083) as well as a very strong negative correlation with the ratio of width to length $X_5$ (–0.9086 ) and the curvature of the ellipse $X_7$ (–0.9187). Due to a strong correlation with several parameters, a strong multicollinearity (linear connection) takes place. This could possibly lead (and will lead, as will be proved below) to the situation when parameter $X_1$ is insignificant and could be ignored during analysis.

It was used ANOVA analysis to build an analytical relationship between the species of oocyst Y and all defined parameters $X_1$–$X_7$. The results are presented in Table 3.

Table 3

Final statistics of ANOVA

| RESULTS | | | | | | |
|---|---|---|---|---|---|---|
| *Regression statistics* | | | | | | |
| Multiple R | 0.9916 | | | | | |
| R-square | 0.9832 | | | | | |
| Adjusted R-square | 0.9826 | | | | | |
| Standard Error | 0.1856 | | | | | |
| Observations | 255 | | | | | |
| Analysis of variance | | | | | | |
| | *df* | *SS* | *MS* | *F* | *Significance F* | |
| Regression | 8 | 495.5088 | 61.9386 | 1797.753 | 1.8E–213 | |
| Residual | 246 | 8.4755 | 0.0345 | | | |
| Total | 254 | 503.9843 | | | | |
| | *Coefficients* | *Standard Error* | *T Stat* | *P–value* | *Lower 95 %* | *Upper 95 %* |
| Y | 74.892 | 12.549 | 5.9675 | 8.35E–09 | 50.17269 | 99.6106 |
| X1 | 0.287 | 0.314 | 0.9128 | 0.36223 | –0.33185 | 0.90512 |
| X2 | 5.527 | 1.491 | 3.7061 | 0.00026 | 2.589843 | 8.46516 |
| X3 | –0.134 | 0.032 | –4.1909 | 3.88E–05 | –0.19719 | –0.0711 |
| X4 | –10.400 | 2.157 | –4.8213 | 2.5E–06 | –14.649 | –6.1514 |
| X5 | –188.590 | 35.298 | –5.3428 | 2.09E–07 | –258.115 | –119.06 |
| X6 | –111.140 | 14.254 | –7.7973 | 1.79E–13 | –139.215 | –83.066 |
| X7 | 513.305 | 110.953 | 4.6263 | 6.02E–06 | 294.7649 | 731.844 |
| X8 | 0.887 | 0.041 | 21.5466 | 1.44E–58 | 0.806174 | 0.96839 |

It was established that parameter $X_1$ (the length of the oocyst) is insignificant according to Student (level of confidence, p<0.36). Accordingly, parameter $X_1$ should be removed from the regression equation. However, this does not mean that the parameter $X_1$ should not be measured at all due to this indicator is indirectly included in the parameter $X_1$–$X_7$. There are the results of the final statistics in Table 4.

Table 4

Final depreciated statistics of ANOVA

| RESULTS | | | | | | |
|---|---|---|---|---|---|---|
| *Regression statistics* | | | | | | |
| Multiple R | 0.991527 | | | | | |
| R-square | 0.983126 | | | | | |
| Adjusted R-square | 0.982648 | | | | | |
| Standard Error | 0.185553 | | | | | |
| Observations | 255 | | | | | |
| Analysis of variance | | | | | | |
| | *df* | *SS* | *MS* | *F* | *Significance F* | |
| Regression | 7 | 495.4801 | 70.78287 | 2055.844 | 5.8338E–215 | |
| Residual | 247 | 8.504231 | 0.03443 | | | |
| Total | 254 | 503.9843 | | | | |
| | *Coefficients* | *Standard Error* | *t Stat* | *p–value* | *Lower 95 %* | *Upper 95 %* |
| Y | 83.463 | 8.323 | 10.02735 | 4.5E–20 | 67.0688 | 99.8571 |
| X2 | 5.721 | 1.476 | 3.876758 | 0.000136 | 2.8145 | 8.6280 |
| X3 | –0.127 | 0.031 | –4.09314 | 5.77E–05 | –0.1881 | –0.0659 |
| X4 | –11.929 | 1.358 | –8.78226 | 2.73E–16 | –14.6050 | –9.2541 |
| X5 | –198.555 | 33.556 | –5.91709 | 1.09E–08 | –264.6483 | –132.462 |
| X6 | –110.535 | 14.233 | –7.76589 | 2.16E–13 | –138.5690 | –82.5005 |
| X7 | 526.497 | 109.971 | 4.787599 | 2.91E–06 | 309.8964 | 743.0970 |
| X8 | 0.886 | 0.041 | 21.53467 | 1.28E–58 | 0.8048 | 0.9668 |

The values in Table 4 demonstrates that the regression equation is reliably significant by the Fisher's criterion, all features and coefficients are significant by Student's criterion ($p$<0.0001), the regression has high value of multiple correlation coefficient (0.9915) and a high value of the coefficient of determination normalized to the number of degrees of freedom (0.9826).

So, the dependence of the species of oocyst Y on the 7 factors identified above is qualitative and could be represented by the following formula:

$$Y=83.463+5.721X_2–0.127X_3–11.929X_4–$$
$$–198.555X_5–110.535X_6+526.497X_7+0.886X_8.$$

The absolute error of the multiple linear regression model is represented as the deviation of the predicted indicator of the species of oocyst between $Y_{pred}$ and the $Y_{fact}$. It was incorrectly identified only 5 of 255 oocyst samples, the relative error in determining the species of Eimeria of turkey < 2 %. Below are the actual values $Y_{fact}$, the predicted values $Y_{pred}$, and the absolute error of the species determination (Table 5). It was represented only five samples in the Table 5 due to the entire dataset is too huge.

Table 5

The results of incorrect determination of oocysts of Eimeria turkeys

| No. sample. | Features | | | | | | | | | | Absolute. Error |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $X_1$, μm | $X_2$, μm | $X_3$, μm $^2$ | $X_4$ | $X_5$ | $X_6$, μm $^{-1}$ | $X_7$, μm $^{-1}$ | $X_8$ | $Y_{fact}$ | $Y_{pred}$ | |
| 20 | 23.82 | 17.60 | 329.25 | 0.674 | 0.739 | 0.154 | 0.062 | 0 | 4 | 3 | 1 |
| 129 | 24.25 | 17.40 | 331.39 | 0.697 | 0.718 | 0.160 | 0.059 | 1 | 5 | 4 | 1 |
| 156 | 23.20 | 17.90 | 326.15 | 0.636 | 0.772 | 0.145 | 0.067 | 1 | 3 | 4 | 1 |
| 159 | 23.25 | 17.80 | 325.03 | 0.643 | 0.766 | 0.147 | 0.066 | 1 | 3 | 4 | 1 |
| 175 | 22.45 | 15.95 | 281.23 | 0.704 | 0.710 | 0.176 | 0.063 | 1 | 3 | 4 | 1 |

**4. Discussion**

Summarizing the results of the research, it should be noted that the regression analysis of morphometric parameters of oocysts is a reliable way to determine the species affiliation of Eimeria pathogens. With our studies the previously proposed methods for the identification of pathogens of oocysts of Eimeria require significant efforts of the researcher to clarify the morphological and biological characteristics of the pathogens and compare them with the descriptive data of identification tables.

The form index has only an ancillary value, as it does not have high specificity for all types of pathogens and does not provide a complete objective assessment [8, 13–15, 21]. The method of Eimeria species identification based on two-dimensional mathematical analysis using an identification index (the ratio of the square of the perimeter of the contour to the surface area of the oocyst [6, 20]

does not fully reflect the morphological features of oocysts as well as the usage of Lagrange interpolation polynomials takes a long time.

Whereas in our work the image obtained on a microscope of oocysts of eimeria is first subjected to morphometry to determine: $X_1$ – the length of the oocyst in μm; $X_2$ – oocyst width in μm and calculate other indicators ($X_3$–$X_7$); $X_8$ – the presence (value 1) or absence (value 0) of the polar bead and automatically using the tool "Regression" from the package "Data Analysis" MS Excel perform analysis and construct multiple linear regression equations to determine the species of oocyst Y. Thus, according to the results of mathematical processing of morphometric dependences of the oocyst structure using the tool "Correlation", a strong correlation was found between the type of oocyst and its length (correlation coefficient 0.9546), area (0.9083) and a strong negative correlation between length to width (–0.9086) and curvature of the ellipse (–0.9187).

**5. Conclusions**

1. It was determined morphological features that are mathematical expressions of morphometric dependences of the structure and identification of the species of oocysts of turkeys.

2. The use of correlation and regression analysis, as well as analysis of variance (ANOVA) allows to reliably identify 98 % of the species affiliation of oocysts of Eymeric turkeys.

**Conflict of interests**

The authors declare that they have no conflicts of interest.

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