Testing the Effectivity of Coccidiostatics by Using Radio Iron-59 for the Quantitative Estimation of Blood in the Caeca and Feces of Chickens

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Abstract. Chickens with Fe 59 labelled erythrocytes were fed with gradated numbers of oocysts of *Eimeria tenella*. The intensity of haemorrhage caused by the infection was determined a) by measuring the activity of the caeca 5 days p. i., b) by measuring the activity of the feces 4—6 days p.i. The statistical evaluation of the results showed the high sensitivity and exactness of both methods used for the testing of the coccidiostatic effect of Ampromium Merck and Nicarbazin Spofa. A 100% effect was obtained with standard doses of Ampromium, an 80% effect with Nicarbazin.

In a previous paper (SCHANZEL 1967) we described a preliminary experiment to confirm the possibility of using radioiron-labelled chicken erythrocytes for proving the amount of haemorrhage in the caeca, caused by *E. tenella*. During studies of Fe 59 administration to mammals, HUFF et al. 1950, 1951, BIRKELAND 1958 a, b, FAUVERT and BOIVIN 1959 a.o. observed the rapid transport of radioiron from the plasma to the erythropoietic tissue and its gradual appearance in the circulating red blood cells. The curve of incorporation into the erythrocytes is exponential, the highest level is reached within 10—14 days. Our experiments confirmed the same course of utilization of radioiron in chickens.

In chickens infected with mature oocysts of *E. tenella*, the first blood generally appears in the feces after 4 days p.i. For our purposes, the most convenient time for infecting the chickens seemed to be the 7th day after the administration of Fe 59 for conducting our measurements at a time, when blood activity was at its maximum.

In these experiments, we attempted to confirm the results of our preliminary experiment, to simplify the methods, to determine the statistical significance of the results obtained and to prove their applicability in the practice. The technical

*) Delivered at a scientific meeting of the Austrian Veterinary Society at the College of Veterinary Medicine in Vienna, March 30, 1967.
**) Technical assistance: J. Medková, J. Šebková,
measurements performed under our conditions are described in another paper (SYNEK and SCHANSEL 1967).

Before starting with the proper work, some preliminary experiments had to be performed:

a) In an experiment with two groups of chickens we found that the curve of Fe 59 utilization by the erythrocytes is the same after an administration into the body cavity and into the vein. In a more numerous group of chickens, this means an essential facilitation and speeding up of work.

b) We confirmed in a simultaneous tracing of the feces activity that parenterally applied radioiron is not expelled from the body. Moderately increased values of feces activity, in some cases observed during the first day p.i., remained statistically insignificant. An experiment with four chickens with surgically separated openings of the urinary and digestive tracts*) showed that plasmatic radioiron is, in fact, expelled with the urine at a time, when its transport to the haematopoietic tissue had not yet been completed. Almost no Fe 59 was expelled with the bile.

c) We traced the activity of feces and blood after a peroral administration of labelled red blood cells both in healthy and *E. tenella* infected chickens. All radioiron applied in this way was found in the feces within the first 48 hrs. The increase in the activity of the blood, as far as this could be confirmed, is statistically insignificant. This shows that in the chicken there is also no confirmable re-resorption of the radioiron from the digestive tract, a fact observed by GEORGI (1964) in sheep.

**METHODS**

In our experiment we used Leghorn-cocks. From the first day of life, the chickens were fed with a standard feeding mixture without an admixture of coccidostatics, antibiotics and vitamins. Radioiron in the form of Fe 59-citrate (Rossendorf) (declared activity 100 μCi/ml to the day of production) was given to 14-day-old chickens. Before use it was diluted in a saline solution and each chicken received a dose of 1–2 μCi in a 1 ml solution. On the 21st day of life the chickens were infected with graded numbers of oocysts of *E. tenella*.

We tried two methods for the tracing of haemorrhages, caused by the maturing schizonts:

1. The chickens were killed 5 days p. i. We took a blood sample, weighed it, measured its activity and calculated the activity in 1 g of blood. We removed both caeca, placed them in test tubes and measured their activity. By converting the obtained value to the activity of one gram of blood it was possible to determine the amount of blood in the caecum in grams.

2. In experiments, where, for various reasons, the chickens had to be kept alive, the activity of a 24 hr-feces collection was determined on the 5th, 6th and 7th day p.i. In this case blood was taken from the elbow vein on the 7th day p.i. The feces were burned to ashes. Sample activity was measured on the bi-crystal gamma spectrometer Tesla NZQ 419. Each sample was measured 10 = 1’.

For the statistical evaluation of the results, we determined the confidence limits. The average mean error, calculated for each experimental group, was multiplied by the value given in the tables by Student for the respective degree of freedom at p = 0.05. The statistical significance of the differences between the groups are illustrated in the figures.

*) We wish to thank Dr. A. Šanda, CSc. from the Department of Fowl-diseases, Veterinary College, Brno, for performing this operation.
By these two described methods we confirmed the effectivity of two coccidiostatic preparations: Amprolium Merck in a dose of 100 mg/kg feeding mixture and Nicarbazin Spofa in 125 mg/kg of feeding mixture.

RESULTS

A. Fig. 1 shows the amount of blood found in the caeca of chickens, killed 5 days after infection. Each group consisted of 10 chickens.

![Graph showing blood content in caeca of chickens infected with oocysts.]

**Fig. 1.** Caeccal blood content in chickens infected with oocysts: A = 10,000, B = 20,000, C = 30,000, D = 40,000, E = control.

![Graph showing blood content in faeces from 4th–6th day after infection with E. tenella.]

**Fig. 2.** Blood content in faeces from 4th–6th day after infection with E. tenella. A = control, B = 5,000 oocysts, C = 10,000 oocysts, D = 20,000 oocysts.

B. Measurements of the feces activity, performed on the 5th, 6th and 7th day p.i. showed that no statistically significant results can be obtained from a quantitative determination of blood in the feces during the individual 24 hr-periods (Tab. 1). These values become highly significant when an addition is made of the blood in the feces, collected during the three critical days (Fig. 2).

<table>
<thead>
<tr>
<th>Number of oocysts per chicken of the group</th>
<th>Average amount of blood/chicken in the feces (in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5th day p.i.</td>
</tr>
<tr>
<td></td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>5,000</td>
<td>0.191</td>
</tr>
<tr>
<td>10,000</td>
<td>0.100</td>
</tr>
<tr>
<td>20,000</td>
<td>4.662</td>
</tr>
<tr>
<td></td>
<td>0.014</td>
</tr>
</tbody>
</table>

Table 1
C. The first experiment with Amprolium Merck was performed with three groups of chickens (10 chickens in each group). From the first day of their life, Amprolium was added to the food. Another 4 groups of chickens were fed without the coccidiostatic admixture. Of these, one group was used for control. Tab. 2 shows the amount of blood in the feces of the chickens during the 72-hr-period of maximum bleeding.

<table>
<thead>
<tr>
<th>Number of oocysts/chicken</th>
<th>Amount of blood in feces/chicken (in g) after 4—6 days p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with Amprolium</td>
</tr>
<tr>
<td></td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>5,000</td>
<td>0.052</td>
</tr>
<tr>
<td>10,000</td>
<td>0.057</td>
</tr>
<tr>
<td>20,000</td>
<td>0.072</td>
</tr>
</tbody>
</table>

D. The second experiment with Amprolium was conducted in the same way, but here, the amount of blood in the caeca of the chickens, killed on the 5th day p.i., was determined. The results of the control group illustrated the content of the blood vessels in the caeca of the noninfected chickens. For statistical evaluation see Fig. 3.

![Fig. 3. Effect of Amprolium on the caecal blood content in E. tenella infected chickens. A = 5,000 oocysts, B = 10,000 oocysts, C = 20,000 oocysts, D = control.](image)

E. Experiments with Nicarbazin Spofa were performed on 10 groups (6 chickens each). We determined the amount of blood in the caeca. The experiment was arranged as follows:

Date of birth of the chickens: Oct. 11, 1966
Administration of Fe 59: Oct. 26, 1966
Infection with E. tenella: Nov. 1, 1966
Nicarbazin applied to group A: Oct. 12—Nov. 6, 1966
B: Nov. 1—Nov. 6, 1966
C: Nov. 2—Nov. 6, 1966
D: Nov. 3—Nov. 6, 1966
E: Nov. 4—Nov. 6, 1966
F: Nov. 2—Nov. 3, 1966
G: Nov. 1, 1966
H: Nov. 1—Nov. 2, 1966
I: control without Nicarbazin, infected
K: control without Nicarbazin, uninfected

Extraction of blood and removal of caeca: Nov. 5, 1966. For results see Fig. 4.

Fig. 4. Effect of Nicarbazin on the caecal blood content in *E. tenella* infected chickens.

Fig. 5. Caecal blood content in by 20,000 *E. tenella* oocysts infected chickens. N = Nicarbazin, A = Amprolium, U = untreated, C = control.

F. Fig. 5 compares the coccidiostatic effect of Nicarbazin Spofa, dose 125 mg/kg of feeding mixture, with the effect of Amprolium Merck, dose 100 mg/kg.

**CONCLUSION AND DISCUSSION**

In the life cycle of *E. tenella*, schizogony is of decisive pathogenic importance. The most marked evidence of this phase is blood in the feces of the infected animals. In studies of the pathogenicity of *E. tenella*, however, other, less noticeable symptoms of the disease are studied: retarded growth, mortality, enlargement of the
caeca, the number of expelled oocysts. These criteria are not very reliable, because they do not consider the primary cause of the disease and the extent of damage done to the caecal lining. Until the present, the amount of blood in the feces of the infected chickens was not considered when estimating the incidence because of the absence of a sensitive and exact quantitative method for determining blood in the feces or in any other biological material.

In view of the results of our previous studies we were in the position to work out two methods, using in both of them Fe 59 labelled erythrocytes, in the one instance for the estimation of the amount of blood in the caeca, in the other for determining the amount of blood in the feces of the infected chickens. Fig. 1 shows that the amount of blood in the caeca is in proportion to the number of oocysts administered to the chickens. The differences between the groups are either highly significant or significant. Tab. 1 shows that no statistically estimable results can be obtained from the quantitative determination of blood in the feces, dropped during the individual 24-hr-intervals. But an addition of the amount of blood in the feces dropped during the three days of maximum bleeding gives values of high statistical significance as shown in Fig. 2. Theoretically it would be more correct to take blood samples not only on the 7th day p.i., but also on the 5th and 6th day p.i. However, the calculation of correction of the disintegration of Fe 59 showed that our error is negligible; there is no doubt that repeated venous puncture would increase it. In both methods we observed very strict regularities in the relation of the number of fed oocysts and the intensity of bleeding. This was partly due to the uniform experimental conditions—origin, breed, age and sex of the chickens, food, temperature, light and other environmental factors, partly to the strict laws governing the asexual reproduction of coccidia. It is, therefore, not surprising that the same numbers of oocysts fed to the chickens were responsible for damaging the same number of cells of the caecal lining and led to the same histological and anatomical changes as described by PELLÉRDY 1965.

A comparison with other methods showed that our methods are not only more exact, but also more time- and labour-saving. Less experimental animals and less oocysts for infection are needed, the chickens have not to be weighed, their caeca have not to be measured and there is also no need to concentrate and count the expelled oocysts. The statistical evaluation is also less complicated because only one factor is being considered.

The suitability of both methods for the practice has been confirmed during the testing of two coccidiostatic preparations. The results of our experiments with Amprolium showed that 100 mg of Amprolium Merck, added to the feeding mixture of chickens, has a 100 % coccidiostatic effect on three-week-old chickens, experimentally infected with 5,000, 10,000 and 20,000 oocysts of E. tenella respectively. In Fig. 4, illustrating our experimental results with Nicarbazin, column A shows the coccidiostatic effect of Nicarbazin, given to the chickens during the experiment, i.e. from the first day of their life. In comparison with the control group (column I), the difference is highly significant. Column B shows that under experimental
conditions, when the chickens cannot be naturally infected, the coccidiostaticum can be given right up to the day of experimental infection; there is no significant difference in the results of group A and B. Following a comparison of the groups B, C, D and E we found that the later the phase of schizogony, encountered by Nicarbazin, the lower its coccidiostatic effect. The differences between the groups are significant to highly significant. A factor decisive for its effectivity was the administration during the first half of the schizogenic phase as evident from column G. In group F, two of the experimental animals died during the experiment, in group H four animals. This fact is responsible for the high deviations in the results. In spite of this, both columns of the figure confirm that the coccidiostaticum was most effective on the first and second day p.i.

In Fig. 5 we compared the coccidiostatic effect of Nicarbazin Spořa with the effect of Amprolium Merck. Under the same conditions, the effect of Amprolium in doses of 100 mg/kg of feeding mixture is a 100 %, while the effect of Nicarbazin in doses of 125 mg/kg of feeding mixture is only about 80 %. MÜLLER and PALKOSKA (1965) and WILLOMITZER (1966) obtained the same results by different methods.

REFERENCES


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