Survey on Drug Resistance of Chicken Coccidia Collected from Japanese Broiler Farms in 1973

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Abstract. A field survey on the drug resistance of chicken coccidia found in Japanese broilers was performed by the chicken inoculation test with 30 fecal samples collected from 29 prefectures in June, 1973. A strain was judged as a resistant one when it showed oocyst production with or without symptoms of coccidiosis in an experimental bird medicated with twice as much as the recommended dose of a coccidiostat.

As a result, 93% and 97% of the strains of *Eimeria acervulina* studied were found resistant to clopidol and decoquinate, respectively. A considerable percentage of the strains of *E. tenella* and *E. necatrix* studied were found resistant to amprolium, clopidol and decoquinate. Only a small percentage of these strains was resistant to sulfadimethoxine. Moreover, multiple drug-resistant strains were found very commonly in *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*.

Distribution of the resistant coccidia was variable over seven geographical districts in Japan.

A large number of *E. acervulina* strains were found resistant to eight times the recommended dose of amprolium and/or decoquinate. Many strains of *E. tenella* and *E. necatrix* were highly resistant to amprolium and decoquinate. Development of resistance to clopidol was variable among these species.

In their previous investigation the present authors [10] surveyed coccidial distribution on Japanese broiler farms in 1973. *Eimeria acervulina* was found to be a predominant species. *E. tenella* decreased in population since a previous survey was made. A large value of OPG (oocysts per gram of feces) was obtained from 80% and 67% of the chickens treated with clopidol and amprolium, respectively.

These results suggested that chicken coccidia might have become less susceptible to the coccidiostats currently used. From this point of view, a field survey was conducted to clarify the actual status of the drug-resistance of chicken coccidia collected from Japanese broiler farms.

**Materials and Methods**

Fecal samples were collected from broiler farms in 29 prefectures (8 districts) of Japan in June (the rainy summer season), 1973. They were the same as those used in the previous study [10].

Oocysts were maintained in a 2% potassium dichromate solution at 25°C to allow to accomplish
sporulation. They were kept alive during the period of the chicken passage test.

Experiment 1

Thirty fecal samples containing oocysts of the tenella, maxima and/or acervulina type were used for the primary test in order to confirm the drug resistance of each type. To obtain fresh oocyst samples for the test, the number of oocysts collected from them to be inoculated into coccidium-free chickens was adjusted to such an extent that the population of fresh oocysts discharged in the feces might be as near as possible to the population in the original fecal sample.

The levels of coccidiosats in the feed were as follows: amprolium, a thiamine derivative, 0.025%; clopidol, a pyridinol derivative, 0.025%; decoquinate, a quinoline derivative, 0.008%; sulfadimethoxine, a sulfa-drug, 0.2% [16].

The chickens used in this test were White Leghorns. They had been reared to be free from coccidia in a sterilized and isolated animal house at the senior author's laboratory.

At 10 days of age, or one day after the initiation of consecutive administration of the coccidial in the feed, groups of five chickens each were inoculated orally with sporulated oocysts. The inoculum size of mixed oocysts of tenella, maxima and acervulina type was decided depending upon the population of oocysts of each type in the original fecal sample.

Unmedicated infected controls were set up for each sample tested to enable identification of the coccidial species.

From 4 to 8 days after inoculation, fecal samples were collected from medicated infected chickens, and examined morphologically to classify discharged oocysts. The prepatent period was also observed in each species.

The clinical findings of infected chickens, especially the character of droppings (bloody or mucous, for example), were also helpful for the diagnosis of the infection.

Three chickens of each group were sacrificed 7 days after inoculation and examined for gross changes of the intestinal tract. In medicated infected chickens without noticeable symptoms, tissue specimens were collected from the anterior, middle and posterior portion of the small intestine, the cecum and the rectum. They were fixed in 10% formalin solution, stained with hematoxylin and eosin, and examined histologically.

In these methods, a strain was judged as resistant when it produced oocysts with or without noticeable symptom of the host.

Experiment 2

The coccidial strains which were contained in eight samples and found to be drug-resistant in Experiment 1 were examined for the degree of resistance to coccidiostats. The tests were carried out with two, four and eight times the recommended dose of each of amprolium, clopidol, decoquinate and sulfadimethoxine.

The animal test was performed in the same way as described in Experiment 1.

Results

Experiment 1

Of the 30 fecal samples collected from the unmedicated infected group 29 were demonstrated by the chicken inoculation to contain oocysts of E. acervulina, 17 of E. maxima, 8 of E. praecox, 27 of E. tenella and 13 of E. necatrix.
SURVEY ON DRUG RESISTANCE OF COCCIDIA IN BROILERS

Table 2. Multiple drug resistance of chicken coccidia collected from broiler farms of Japan in June, 1973

<table>
<thead>
<tr>
<th>Species</th>
<th>Susceptible (%)</th>
<th>Resistant to 1 drug</th>
<th>2 drugs</th>
<th>3 drugs</th>
<th>4 drugs</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. acervulina</em></td>
<td>1/29 (3)</td>
<td>0 (C)</td>
<td>21 (C, D: 20)</td>
<td>7 (C, D, S)</td>
<td>— (D)</td>
<td>28/29 (97)</td>
</tr>
<tr>
<td><em>E. maxima</em></td>
<td>5/17 (30)</td>
<td>0</td>
<td>12 (C, D)</td>
<td></td>
<td>—</td>
<td>12/17 (71)</td>
</tr>
<tr>
<td><em>E. praecox</em></td>
<td>1/8 (13)</td>
<td>5 (C)</td>
<td>2 (C, D)</td>
<td></td>
<td>—</td>
<td>7/8 (88)</td>
</tr>
<tr>
<td><em>E. tenella</em></td>
<td>11/27 (41)</td>
<td>3 (A)</td>
<td>8 (A, C: 6)</td>
<td>4 (A, C, D)</td>
<td>1 (A, C, D, S)</td>
<td>16/27 (59)</td>
</tr>
<tr>
<td><em>E. necatrix</em></td>
<td>9/13 (69)</td>
<td>0</td>
<td>2 (A, C: 1)</td>
<td></td>
<td>2</td>
<td>4/13 (31)</td>
</tr>
</tbody>
</table>

Remarks:
A: Amprolium (0.02% in feed), C: Clopidol (0.025%), D: Decoquinate (0.008%) and S: Sulfadimethoxine (0.2%).
a: Not tested against amprolium.

Table 3. Regional distribution of drug-resistant chicken coccidia collected from broiler farms of Japan in June, 1973

<table>
<thead>
<tr>
<th>District</th>
<th>No. of strains used</th>
<th>Amprolium(%)</th>
<th>Clopidol</th>
<th>Decoquinate</th>
<th>Sulfadimethoxine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido &amp; Tohoku</td>
<td>6</td>
<td>2/3 (67)</td>
<td>2/6 (33)</td>
<td>3/6 (50)</td>
<td>1/6 (17)</td>
</tr>
<tr>
<td>Kanto</td>
<td>17</td>
<td>3/8 (38)</td>
<td>7/12 (80)</td>
<td>8/12 (63)</td>
<td>1/12 (8)</td>
</tr>
<tr>
<td>Chubu</td>
<td>15</td>
<td>3/6 (50)</td>
<td>12/15 (80)</td>
<td>9/15 (60)</td>
<td>2/15 (13)</td>
</tr>
<tr>
<td>Kinki</td>
<td>16</td>
<td>4/6 (67)</td>
<td>13/16 (81)</td>
<td>10/16 (63)</td>
<td>2/16 (12)</td>
</tr>
<tr>
<td>Chugoku</td>
<td>15</td>
<td>1/7 (14)</td>
<td>7/15 (47)</td>
<td>7/15 (47)</td>
<td>1/15 (7)</td>
</tr>
<tr>
<td>Shikoku</td>
<td>9</td>
<td>2/3 (67)</td>
<td>7/9 (78)</td>
<td>6/9 (67)</td>
<td>3/9 (33)</td>
</tr>
<tr>
<td>Kyushu</td>
<td>16</td>
<td>5/7 (71)</td>
<td>12/16 (75)</td>
<td>8/16 (50)</td>
<td>2/16 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>20/40 (50)</td>
<td>60/94 (64)</td>
<td>51/94 (54)</td>
<td>12/94 (13)</td>
<td></td>
</tr>
</tbody>
</table>

Remarks:
a: Tested against *E. tenella* and *E. necatrix* only.
b: Number of resistant strains/Number of strains tested.

Field strains of *E. acervulina* showed a high rate of resistance to clopidol (93% of the tested) and decoquinate (97%), but a low rate of resistance to sulfadimethoxine (28%). *E. maxima* and *E. praecox* were resistant to clopidol and decoquinate at fairly high rates. *E. tenella* was resistant to amprolium, clopidol, decoquinate and sulfadimethoxine at considerable rates, but was less resistant to these substances than *E. acervulina*.

These results are summarized in Table 1. The majority of drug-resistant strains were resistant simultaneously to two or more coccidiostats. They were called multiple drug-resistant strains (Table 2).

The regional distribution of drug-resistant strains is shown in Table 3. The distribution among 7 geographical districts was clarified by the analysis of variance of frequency type [18]. The detection rate of resistant strains was significantly lower in the Kanto and Chugoku districts than in any other district at a 5% level. In every district the distribution of strains resistant to sulfadimethoxine was significantly more
Limitation on the resistance of strains resistant to any other coccidiosis is

Experiment 2

The coccidial strains derived from eight samples and found resistant to various coccidiosis in Experiment 1 were examined for the degree of resistance to the respective drugs. The results obtained are shown in Table 4.

Most of the strains of *E. acervulina* identified were resistant to amprolium and decoquinate at over eight times the recommended level. Most of the strains of *E. tenella* identified were highly resistant to amprolium. *E. maxima* was found to be more highly resistant to decoquinate than to clopidol. Some strains of *E. acervulina* were reduced in susceptibility to sulfadimethoxine, but *E. tenella* and *E. maxima* were susceptible to the drug.

Development of drug resistance was found to be different among the Eimeria species collected from the same fecal sample, although these species were thought to have been exposed to the same coccidiosis for the same time in the field.

Discussion

The appearance of drug-resistant strain of chicken coccidia in Japan was first reported by Tsuoda [14, 15]. In 1961 he isolated in Tokyo several strains of *E. tenella* which were resistant to sulfa-drugs excepting sulfadimethoxine.

Itoh [2] also isolated sulfadrug-resistant cecal coccidia in chickens collected from a poultry farm in Aichi Prefecture, though intestinal coccidia collected in the same chickens were susceptible to the drug.

Since then poultry industry has developed rapidly in Japan and large-scale floor feeding has become popularized. Accordingly, damage by coccidiosis is now a serious problem in this country.

Amprolium was highly effective against cecal coccidiosis and has been widely used in Japan for a long period of time. It has displayed little side effect on both chicken and man, and its medication has hardly induced a resistant strain.
With an increase in the population of intestinal coccidia, amprolium plus ethopabate and several quinoline derivatives were introduced into poultry farms. The efficacy of the quinoline derivatives quickly declined due to the sudden appearance of resistant coccidia. Amprolium has shown a decrease in efficacy against E. tenella and E. necatrix found on poultry farms.

When clopidol, a broad-spectrum coccidiostat, was introduced, it took the place of amprolium as top agent. A trend like this had already been suggested from the results of the present authors' surveys in 1973 [10]. In May, 1973, clopidol was used on 45 farms (75% of those surveyed) and amprolium on 15 farms (18%). On the other hand, these drugs were used on 75 farms (67%) and 4 farms (3%), respectively, in June.

Some strains of E. tenella and E. necatrix increased in resistance to amprolium, as is shown in Tables 1 and 4. Although quinoline derivatives were used only for a short time, resistance to decoquinate was detected at a high rate, as is clear from Table 1. It seemed that quinoline derivatives might be liable to give rise to a resistant strain in the field, as was suggested in the papers on experimental inducement of resistance to decoquinate [9, 12].

Clopidol has widely used on Japanese poultry farms since 1971. As indicated above, it was the main coccidiostat in use in 1973 when the present surveys were conducted. Tsunoda et al. [17, 18] and Ikeda et al. [1], however, reported that the strains of E. acervulina isolated from Japanese poultry farms in 1973 decreased in sensitivity to clopidol. The present authors [10] detected coccidial oocysts at a high OPG value from 86% of 72 fecal samples collected from clopidol users in June, 1973. In present survey, E. acervulina was found to be extremely highly resistant to clopidol even when eight times the recommended dose of this drug was used.

In the previous surveys [10], degenerated oocysts were detected at a low OPG value from fecal samples collected from farms of sulfa-drug users. Sulfa-drugs have been used for 20 years and more than 10 years have passed since the appearance of sulfa-drug resistant strains of E. tenella was reported. Coccidia resistant to sulfa-drugs are not so common as those resistant to pyridinol, thiamine or quinoline derivatives. It is supposed to be one reason for this that sulfa-drugs are administered intermittently as therapeutics, while the other coccidiostats are given continually with feed for prophylactic purposes.

Methods used by various researchers for the assessment of drug resistance differ from one another [19]. The most commonly used points for assessment are oocyst production and body weight gains.

McLoughlin [8] used an anticoccidial index to measure drug resistance in a series of studies on E. tenella. The index was calculated from oocyst count, fatality, weight gain, and lesion score of the intestinal tissue.

Jeffers [3–5] used a criterion of resistance which was defined as the average dropping score of the medicated group, this score being at least 50% of that of the unmedicated control group.

Ryley [11] suggested that oocyst production might be a very unreliable quantitative criterion of coccidial infection. He preferred weight gain and/or fatality to oocyst production to assess the response to a drug.

from American broiler farms.

In the present study, some difficulties were found in assessment of the drug resistance of field samples of intestinal coccidia of such species as *E. acervulina*, *E. maxima* and *E. praecox*. These species could not clearly be discriminated from one another by the intestinal lesion score or the dropping score. So that, detection of oocysts and prepatent period were key factors for discrimination of these species.

The criterion set up for the resistance of a coccidial strain to coccidiostats in this study was as follows: A strain was judged as “resistant” when oocysts were detected with or without noticeable fecal changes or intestinal lesions in animals administered with two times the recommended dose of the drug. In the preliminary examination of *E. tenella* infection, a strain judged as “resistant” under this criterion may generally correspond to a strain of “reduced sensitivity” and/or “resistant” strain reported as such by Jeffers [6] according to a criterion established by him on the basis of the recommended dose of a drug.

References


