Use of microscopic lesion scores, gross lesion scores and oocyst count scores to detect Eimeria maxima in chickens

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Use of microscopic lesion scores, gross lesion scores and oocyst count scores to detect *Eimeria maxima* in chickens

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We recently found that the microscopic lesion scoring (MLS) method is superior to gross lesion scores (GLS) for detecting endogenous stages of *Eimeria maxima* in chickens. In the present study, the MLS was found to be superior to either oocyst count scoring (OCS) or GLS for detecting *E. maxima* infections in broiler chickens, and the OCS was better than the GLS. For chickens in all companies and at all geographic locations, the MLS located more *E. maxima* than did the OCS or the GLS.

Though used widely and routinely, the GLS does not detect *E. maxima* that can easily be located with other methods. Therefore, the GLS may not be suitable for use in health programmes that depend upon detection of *E. maxima* in broiler chickens.

**Introduction**

While *Eimeria* spp. infections in chickens often adversely affect performance and incur expense (McDougald & Reid, 1991), prevention of *Eimeria* infections is also costly. Either gross lesion scores (GLS) or oocyst count scores (OCS) are used to measure *Eimeria* infection (McDougald & Reid, 1991). The score data usually are used to help poultry company personnel judge *Eimeria* infections, and decide if infections are being satisfactorily controlled. If coccidiosis control is perceived to be unsatisfactory, then changes are made to the coccidiosis control programme.

Traditionally, GLS or OCS have been used to measure and monitor *Eimeria* infection (Johnson & Reid, 1970; McDougald & Reid, 1991). In previous investigations, we have found that the microscopic lesion scoring (MLS) method is superior to GLS for detecting *Eimeria maxima* infections in chickens (Idris et al., 1996a,b, 1997a, b; Brown & Goodwin, 1997; Goodwin et al., 1997a, b). However, the relationship between MLS and OCS for locating *E. maxima* in chickens has not been investigated. The purpose of the present study was to perform such an investigation.

**Materials and Methods**

**Chickens, specimens, and scores of *E. maxima* infection**

Score data were collected from 900 broiler chickens that were grown on 175 farms that produce chickens for eight companies at 17 locations across the southern United States. For each chicken, a GLS, an OCS, and a MLS were determined (paired data). In all companies, examination of these chickens was random and was performed as part of each producer's routine coccidiosis monitoring programme.

Chickens were humanely killed by either CO2 inhalation or cerebrocervical dislocation. Samples from each chicken were assigned a unique number and examined for *E. maxima*. Scores were tabulated in a database in a microcomputer.

**Gross lesion scores.** For all GLS, the method of Johnson & Reid (1970) was used by one of us (KD). GLS ranged from 0 (no gross lesions) to 4 (most severe gross lesions).

**Oocyst count scores.** A 10X objective lens was used for all OCS and MLS. For all OCS, the direct smear method was used by one of us (KD). A solitary smear was prepared from the jejunal mucosa, and numbers of *E. maxima* oocytes only were counted, and scores were assigned where 0 = no oocysts seen, 1 = 1 to 20 oocysts per ×10 (objective) field, 2 = 21 to 50 oocyst per ×10 field, 3 = 51 to 100 oocysts per ×10 field, and 4 = numbers of oocysts per ×10 field were too numerous to count.

**Microscopic lesion scores.** For all MLS, a 2.5 cm long portion of jejunum proximal to Meckel's diverticulum was collected, opened,
and immersed in coded jars filled with 10% neutral buffered formalin. The volume of fixative-to-organ exceeded 10:1. Portions of each intestine segment were cut parallel to their longitudinal axis, placed into coded cassettes, processed through graded ethanol and xylene, and embedded in paraffin. Sections (3 μm) of deparaffinized formalin-fixed paraffin-embedded intestine were placed onto glass slides, stained with hematoxylin and eosin, and examined by one pathologist (MAG) using a light microscope. Diagnosis of *E. maxima* was based upon finding characteristic developmental stages of this parasite (Goodwin, 1996). The MLS is the sum of A plus B where A represents the distribution of developmental stages of *Eimeria* along the examined intestine segment (0 = no parasites, 1 = parasites in one X 10 field, 2 = parasites in two X 10 fields, 3 = parasites in three X 10 fields, 4 = parasites in all four X 10 fields), and B represents the severity of *Eimeria* infection within the examined fields (0 = parasites in 0% of villi, 1 = parasites in <25% of villi, 2 = parasites in 25 to 50% of villi, 3 = parasites in 51 to 75% of villi, 4 = parasites in >75% of villi). For example, if A = 2 (parasites in two X 10 fields) and B = 2 (parasites in 25 to 50% of villi), then A + B = 4. The initial total MLS could range from 0 to 8. For biometric comparisons of GLS and OCS to MLS, the MLS was divided by 2 (thus, the final MLS ranged from 0 to 4).

**Biometrics**

The data were analysed using a two-factor analysis of variance. Factor 1 was the type of scoring system, and factor 2 was the farm. Test type and farm findings were further analyzed using a Tukey multiple comparison test. The frequency data for the three methods were analyzed using a chi-square: one-way analysis of variance. Multiple comparison among the proportions were calculated using a Tukey multiple comparison test.

**Results**

In the present study, significant ($P < 0.001$) differences were found among methods used to detect *E. maxima* infections in broiler chickens on 175 farms. The MLS was superior to either the OCS or the GLS. For all companies at all locations, the MLS located significantly ($P < 0.001$) more *E. maxima* than did the OCS or the GLS. For brevity, not all findings for the 175 farms that grew chickens for the eight companies at 17 locations are published here. The observed outcomes for the three methods of scoring score *E. maxima* infections in chickens on nine farms that grew chickens for one company at one location are shown in Table 1. The frequencies of significant outcomes for the three methods are summarized in Table 2. Chi-square one-way analysis of variance is highly significant ($F = 37.97$, $df = 5/1044$, $P < 0.001$) for all outcomes except GLS > MLS versus GLS > OCS. Cumulative scores for the three methods used to measure *E. maxima* infections in all chickens in the present study are illustrated in Figure 1.

**Discussion**

The MLS is an objective measure of *E. maxima* infection in broiler chickens. Furthermore, for chickens tested in health programmes in any company, at any complex location, or on any farm as in the present study, the MLS will identify the presence of more *E. maxima* than will either the GLS or OCS. Therefore, under commercial conditions the MLS is superior to OCS and GLS for detecting *E. maxima* infection. Although the OCS is inferior to MLS for detecting *E. maxima*, the OCS is superior to the GLS. The GLS does not detect *E. maxima* that can be located with other methods. Therefore, the GLS does not appear suit-

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**Table 1. Comparison of three types of tests** for *Eimeria maxima* in broilers on each of nine farms in one company at one location

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>2</td>
<td>38.86</td>
<td>19.43</td>
<td>11.49 ($P &lt; 0.001$)</td>
</tr>
<tr>
<td>Farm</td>
<td>8</td>
<td>22.01</td>
<td>2.75</td>
<td>1.63 n.s.</td>
</tr>
<tr>
<td>Type × Farm</td>
<td>16</td>
<td>14.53</td>
<td>0.908</td>
<td>0.54 n.s.</td>
</tr>
<tr>
<td>Error</td>
<td>45</td>
<td>76.08</td>
<td>1.69</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 2. Frequency of significant outcomes of three methods used to detect *Eimeria maxima* in broilers**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLS &gt; GLS</td>
<td>70</td>
</tr>
<tr>
<td>OCS &gt; GLS</td>
<td>56</td>
</tr>
<tr>
<td>MLS &gt; OCS</td>
<td>9</td>
</tr>
<tr>
<td>GLS &gt; MLS</td>
<td>0</td>
</tr>
<tr>
<td>GLS &gt; OCS</td>
<td>0</td>
</tr>
</tbody>
</table>

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1Within a farm (row), any test type with the same superscript is not significantly different at the 5% level of probability (0.05).
2Within a scoring type (column), farms with the same subscript are not significantly different at the 5% level of probability (0.05).
3OCS = oocyst count score, GLS = gross lesion score, MLS = microscopic lesion score.

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1Outcomes were considered different when test scores were found to be significantly different. Chi-square: one-way analysis of variance is highly significant ($F = 37.97$, $df = 5/1044$, $P < 0.001$) for all outcomes except GLS > MLS versus GLS > OCS.
able for use in health programmes that depend upon detection of *E. maxima* in broiler chickens.

The results from the present study, are not surprising, since more can be seen by microscopy than with the unaided eye. There are added benefits to MLS testing namely that light microscopy detects not only oocysts but also developmental stages of the parasite, and use of the microscope provides an opportunity for recognition of other causes of intestinal disease. (Goodwin, 1996).

Acknowledgements

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References


Three methods to measure *E. maxima* infection


Résumé

Utilisation des notations des lésions microscopiques, macro- scopiques et comptages des oocystes pour détecter *Eimeria maxima* chez le poulet

Il a été récemment montré que la méthode de notation des lésions microscopiques (MLS) est supérieure à celle de la notation des lésions macroscopiques (GLS) pour détecter les stades endogènes d’*Eimeria maxima* chez les poulets. Dans cette étude, la MLS a été supérieure au comptage des oocystes (OCS) ou à la GLS pour la détection des infections à *E. maxima* chez les poulets de chair, et le OCS est supérieur à la GLS. Pour les poulets des différents types commerciaux et répartis dans toutes les zones géographiques, la MLS localise mieux *E. maxima* que le fait le OCS ou la GLS.

Bien qu’utilisée routine et à grande échelle, la GLS ne détecte pas *E. maxima* qui peut être localisée par d’autres méthodes. En conséquence, la GLS ne paraît pas souhaitable à employer dans les programmes visant la détection d’*E. maxima* chez les poulets de chair.

Zusammenfassung

Verwendung von Scores für die mikroskopischen Läsionen, makroskopischen Läsionen und Oozyestenzahlen zum Nachweis von *Eimeria maxima* bei Hühnern


Obwohl das GLS-Verfahren in weiten Kreisen und routinemäßig verwendet wird, läßt sich *E. maxima*, die mit anderen Methoden leicht ausfindig gemacht werden kann, damit nicht nachweisen. Das GLS-Verfahren dürfte deshalb nicht für die Verwendung in Gesundheits- sprogrammen geeignet sein, bei denen es auf den Nachweis von *E. maxima* in Mastküken ankommt.

Resumen

Uso de la evaluación de las lesiones microscópicas, lesiones macro- scopicas y recuento de ooquistes para la detección de *Eimeria maxima* en pollos

Hemos descubierto recientemente que la utilidad del método de evaluación de lesiones microscópicas (MLS) es más eficaz que el método de evaluación de lesiones macroscópicas (GLS), para la detección de los estudios endógenos de *Eimeria maxima* en pollos. En este estudio, el MLS demostró ser más efectivo que el recuento de ooquistes (OCS) o el GLS para la detección de infestaciones de *Eimeria maxima* en broilers; y el OCS fue más efectivo que el GLS.
En pollos de diferentes empresas y áreas geográficas el MLS detectó más *Eimeria maxima* que el OCS y el GLS.

Aunque el GLS se usa ampliamente y rutinariamente, no es capaz de detectar *Eimeria maxima* con tanta facilidad como lo hacen los otros métodos. Por ello el GLS puede que no sea adecuado para la utilización en programas de salud que dependen de la detección de *Eimeria maxima* en pollos.