**Mycoplasma gallisepticum**: influence of cell invasiveness on the outcome of experimental infection in chickens

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Abstract

Recently we have shown that a low (Rlow) and a high laboratory passage (Rhigh) of the poultry pathogen *Mycoplasma gallisepticum* prototype strain R differ markedly in their capability to invade non-phagocytic eukaryotic cells. In the present study the infection traits of these two mycoplasma passages were compared in an in vivo setting. After aerosol inoculation of chickens, *M. gallisepticum* was re-isolated from the inner organs of birds infected with Rlow, whereas no mycoplasma was recovered from the inner organs of birds infected with Rhigh. These results indicate that the two mycoplasma populations derived from strain R differ in their capacity to cross the mucosal barrier and suggest that cell invasion may play a major role in the observed systemic spreading of *M. gallisepticum* in its chicken host.

Keywords: Systemic infection; Cell invasion; Host colonization; Experimental infection; Respiratory disease; *Mycoplasma*

1. Introduction

The *Mycoplasma* genus comprises over 100 species that represent wall-less prokaryotes, some of which are known to cause chronic diseases in man and animals. The avian pathogen *Mycoplasma gallisepticum* induces severe chronic respiratory disease in chickens [1] as well as sinusitis in turkeys [2]. These diseases (for review see [3]) are globally prevalent and economically damaging to the poultry industry worldwide due to their effects on feed efficiency [4]. Although *M. gallisepticum* infections mainly affect the respiratory tract, they may occasionally cause keratoconjunctivitis [5], salpingitis [6,7], arthritis [8] and fatal encephalopathy [9]. The avian pathogen can be transmitted from infected birds via aerosol or via the egg [10]. Apart from a small number of reports describing the isolation of *M. gallisepticum* after intravenous inoculation from the hock of arthritic chickens [6] or from the brain of turkeys [9,11] and from the bile [12] or the brain [13] of naturally infected birds, the presence of *M. gallisepticum* in various inner organs has never been assessed. In birds experimentally infected via the respiratory tract, such findings would formally demonstrate that *M. gallisepticum* has the capability to translocate across the respiratory mucosal barrier, enter the bloodstream and disseminate throughout the body.

The virulence factors that promote *M. gallisepticum* infection and induce disease are poorly understood and are most likely influenced by the host and the environment. Earlier studies revealed that *M. gallisepticum* strains differ markedly in their pathogenicity for chickens [10,14,15] and that in vitro passages in culture medium of a particular *M. gallisepticum* strain [16] affect its virulence [14]. More specifically, the evaluation of the pathogenic effects of *M. gallisepticum* on the respiratory tract by experimental infections of chickens revealed that a low (Rlow) as well as a high laboratory passage (Rhigh) of the *M. gallisepticum* prototype strain R both colonize the trachea, while only Rlow induces air sac lesions [14]. Recently, we have shown that these two passages also differ in their ability to invade non-phagocytic eukaryotic cells in vitro: while Rlow was capable of establishing intracellular residence, the high passage, Rhigh, did not [17]. In that previous study it was
also shown that 10 times passaging of R\( _{\text{high}} \) through cell culture led to an enrichment of organisms that have retained the invasive phenotype which is characteristic of R\( _{\text{low}} \). These findings raised the question of whether R\( _{\text{low}} \) and R\( _{\text{high}} \) would also differ in their ability to cross the mucosal barrier and to spread throughout the body. In this study, we have examined the presence of mycoplasmas in samples collected from the respiratory tract and from various inner organs of birds inoculated via aerosol with either M. galls\( _{\text{epistic}} \) R\( _{\text{low}} \), R\( _{\text{high}} \) or R\( _{\text{high}} \)P10 and have assessed the clinical signs and pathological lesions induced by these three populations.

2. Materials and methods

2.1. Mycoplasma strains and growth condition

M. galls\( _{\text{epistic}} \) laboratory passages R\( _{\text{low}} \) and R\( _{\text{high}} \) used in this study were kindly provided by S. Levisohn, Kimron Veterinary Institute, Bet Dagan, Israel. R\( _{\text{low}} \) and R\( _{\text{high}} \) correspond to the prototype strain R propagated 10 times in culture medium, respectively [16]. The population designated R\( _{\text{high}} \)P10 was previously obtained by propagating R\( _{\text{high}} \) 10 times in Hayflick medium [17]. Prior to infection, mycoplasma cultures were grown at 37°C in modified Hayflick medium [18] containing 20% (v/v) heat-inactivated horse serum (Life Technologies, Rockville, MD, USA) to mid-exponential phase, as indicated by the metabolic color change of the medium. The number of viable mycoplasmas in a suspension was determined by plating serial dilutions on Hayflick medium containing 1% (w/v) agar, followed by incubation at 37°C. After 6–8 days, the number of colony forming units (CFU) was counted using an SMZ-U stereomicroscope (Nikon, Tokyo, Japan).

2.2. Animal experiments

2.2.1. Selection of mycoplasma-free chickens

One hundred and twenty 1-day-old Arbor across chickens were selected from a flock certified free of M. galls\( _{\text{epistic}} \) by serological examination using (i) the rapid slide agglutination (RSA) test with stained M. galls\( _{\text{epistic}} \) antigens (Antigen Nobilis, Intervet International, Boxmeer, The Netherlands), and (ii) a monoclonal antibody-based blocking ELISA (MYGA test, Diagnosztikum, Budapest, Hungary) [19]. The parental flock was also certified M. synoviae-free by standard serological examination using the M. synoviae Intervet Antigen Nobilis.

Twelve 1-day-old chicks from the birds selected for infection studies were slaughtered and examined for pathological lesions as well as for the presence of mycoplasmas. No pathological lesions characteristic of mycoplasma infection were found and cultivation for mycoplasmas was negative. Sera collected from these chickens were shown to be free of maternal antibodies to M. galls\( _{\text{epistic}} \) and M. synoviae using the two tests mentioned above.

2.2.2. Experimental infection procedure

At the age of 21 days, the 108 remaining chickens were weighed, marked and divided into four groups of 27 chickens so that the average body weight of each group did not significantly differ based on Student’s t-test. Each group was placed into an aerosol chamber of 0.224 m\(^3\) and inoculated respectively with 5 ml of culture medium containing (i) \( 1 \times 10^9 \) CFU ml\(^{-1} \) of R\( _{\text{high}} \), (ii) \( 0.83 \times 10^9 \) CFU ml\(^{-1} \) of R\( _{\text{low}} \), (iii) \( 0.99 \times 10^9 \) CFU ml\(^{-1} \) of R\( _{\text{high}} \)P10, or (iv) no mycoplasmas. Each inoculum was pulverized into fine aerosol particles of 7–10 μm and sprayed for 2 min into the chamber. Birds were maintained in the unventilated aerosol chamber for an additional 15 min before they were transferred to their isolation units. Feeding and cleaning of the four groups were then performed by four individual crews to avoid risks of cross-contamination. Birds were then subjected to daily clinical examination and after 3, 6 and 9 days, nine birds of each group were weighed, slaughtered, and necropsy was performed for pathomorphological lesions. The lesions were documented by a scoring system characterized by the amount of fibrinous exudate on the serous membrane of the thoracic air sacs. Gross lesions were scored on a scale from 0 (no lesions) to 8 (severe bilateral lesions) [20]. During necropsy, swabs were collected from (i) the trachea, (ii) the lung, (iii) the left air sac, (iv) the liver, (v) the spleen, (vi) the kidney, (vii) the brain and (viii) the heart and directly seeded into 5 ml of liquid medium. After 3 and 6 days of growth, the metabolic color change of each culture was recorded and an aliquot was plated onto solid medium to monitor the presence or absence of mycoplasmas. To confirm that mycoplasmas recovered from the samples of infected birds were indeed M. galls\( _{\text{epistic}} \), 80 positive and negative cultures were randomly selected and subjected to polymerase chain reaction (PCR) using a protocol previously described [21].

2.3. Statistical analysis

The number of birds presenting air sacculitis and the frequency of re-isolation of M. galls\( _{\text{epistic}} \) from the respiratory tract and from inner organs were analyzed by the chi-square test using the SPSS 9.0 (SPSS Incorporation) software \((n = 27)\). The probability level for significance was \( P \leq 0.05 \).

3. Results

In order to define the capability of M. galls\( _{\text{epistic}} \) to generate a systemic infection, the presence of the avian pathogen was assessed in samples collected from the re-
infected with R\textsubscript{high} and 0.5I\textsubscript{2}VB0.54 and 0.89I\textsubscript{2}VB0.33 in birds corresponding values are 0.25I\textsubscript{2}VB0.35 and 0.33I\textsubscript{2}VB1 in birds in-
0.63I\textsubscript{2}VB0.52 at day 3 and 5.22I\textsubscript{2}VB1.72 at day 9 (the corre-
3.1. Clinical and pathological observations in experimentally infected chickens

At day 6, the group infected with R\textsubscript{low} started showing signs of respiratory distress such as tracheal rales, sneezing and coughing which intensified until the last birds were killed at day 9. None of these signs was observed in birds infected with R\textsubscript{high}, or with R\textsubscript{highP10}, or in those that had only received medium.

During necropsy, no air sac lesion was found in birds of the control group (Table 1). In the group infected with R\textsubscript{low}, 24 out of the 27 birds showed air sac lesions in contrast to only two of the 27 birds that composed the group infected with R\textsubscript{high}. Interestingly, 18 out of 27 birds of the group infected with R\textsubscript{highP10} also showed air sacculitis. Even though the number of birds presenting air sacculitis is significantly higher (P \leq 0.001) in birds infected with R\textsubscript{high}.

3.2. Serological response

Blood samples were collected following necropsy at day 3, 6 and 9, and the presence of host antibodies to M. gallisepticum was assessed by RSA. As illustrated in Table 2, sera collected from birds infected with R\textsubscript{high} did not contain any detectable antibodies to M. gallisepticum throughout the experiment, while RSA-positive reactors were observed at day 6 in groups infected with R\textsubscript{low} (three out of nine serum samples) and R\textsubscript{highP10} (one out of nine serum samples). At day 9, the assay was positive for all remaining birds infected with R\textsubscript{low} and for three out of the nine remaining birds infected with R\textsubscript{highP10}.

3.3. Re-isolation of M. gallisepticum from the respiratory tract

Swabs were collected during necropsy from the trachea, the lungs and from the left thoracic air sac of each bird and directly transferred into liquid medium. Cultures were plated at days 3 and 6 following inoculation. No mycoplasma colony was observed on plates seeded with liquid culture that did not present a metabolic color change. The identity of M. gallisepticum was confirmed by PCR on randomly selected positive cultures which resulted in a single PCR product of the expected size using a primer set previously described [21], while randomly selected negative cultures did not generate a detectable amplification. As illustrated in Table 3, the presence of mycoplasmas was only detected in the three infected groups although mycoplasmas were not re-isolated from all birds of the same group. Also, re-isolation of mycoplasmas from one organ of the respiratory tract of a given bird did not imply the recovery of the pathogen from the other two sites (Table 4). For instance, re-isolation of M. gallisepticum from inner organs.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Total number of birds with air sacculitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsubscript{high}</td>
<td>0/9 (0)\textsuperscript{a}</td>
<td>1/9 (1)</td>
<td>1/9 (3)</td>
<td>2/27 (4)\textsuperscript{b}</td>
</tr>
<tr>
<td>R\textsubscript{low}</td>
<td>6/9 (7)</td>
<td>9/9 (28)</td>
<td>9/9 (47)</td>
<td>24/27\textsuperscript{c} (82)</td>
</tr>
<tr>
<td>R\textsubscript{highP10}</td>
<td>4/9 (4)</td>
<td>6/9 (6)</td>
<td>8/9 (8)</td>
<td>18/27\textsuperscript{d} (66)</td>
</tr>
<tr>
<td>Control</td>
<td>0/9 (0)</td>
<td>0/9 (0)</td>
<td>0/9 (0)</td>
<td>0/27 (0)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Lesion scores per group.
\textsuperscript{b}Total lesion scores.
\textsuperscript{c}The number of birds presenting air sacculitis is significantly higher in birds infected with R\textsubscript{low} than in birds infected with R\textsubscript{high} (P \leq 0.001).
\textsuperscript{d}The number of birds presenting air sacculitis is significantly higher (P \leq 0.001) in birds infected with R\textsubscript{highP10} than in birds infected with R\textsubscript{high}.

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Total reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>R\textsubscript{high}</td>
<td>0/9\textsuperscript{e}</td>
<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
<tr>
<td>R\textsubscript{low}</td>
<td>0/9</td>
<td>3/9</td>
<td>9/9</td>
<td>12/27</td>
</tr>
<tr>
<td>R\textsubscript{highP10}</td>
<td>0/9</td>
<td>1/9</td>
<td>3/9</td>
<td>4/27</td>
</tr>
<tr>
<td>Control</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
</tbody>
</table>

\textsuperscript{e}Number of positive birds/total number of birds.

M. gallisepticum was assessed by RSA. As illustrated in Table 2, sera collected from birds infected with R\textsubscript{high} did not contain any detectable antibodies to M. gallisepticum throughout the experiment, while RSA-positive reactors were observed at day 6 in groups infected with R\textsubscript{low} (three out of nine serum samples) and R\textsubscript{highP10} (one out of nine serum samples). At day 9, the assay was positive for all remaining birds infected with R\textsubscript{low} and for three out of the nine remaining birds infected with R\textsubscript{highP10}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Inoculum & Day 3 & Day 6 & Day 9 & Total reactors \\
\hline
R\textsubscript{high} & 0/9\textsuperscript{e} & 0/9 & 0/9 & 0/27 \\
R\textsubscript{low} & 0/9 & 3/9 & 9/9 & 12/27 \\
R\textsubscript{highP10} & 0/9 & 1/9 & 3/9 & 4/27 \\
Control & 0/9 & 0/9 & 0/9 & 0/27 \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Inoculum & Day 3 & Day 6 & Day 9 \\
\hline
R\textsubscript{high} & 4/9\textsuperscript{c} & 0/9 & 8/9 \\
R\textsubscript{low} & 6/9 & 9/9 & 9/9 \\
R\textsubscript{highP10} & 6/9 & 8/9 & 8/9 \\
Control & 0/9 & 0/9 & 0/9 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{c}Number of positive birds/total number of birds.

\textsuperscript{d}The frequency of re-isolation of M. gallisepticum from inner organs of birds infected with R\textsubscript{low} is signiﬁcantly higher (P \leq 0.001) than in birds infected with R\textsubscript{high}.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
Inoculum & Day 3 & Day 6 & Day 9 \\
\hline
RT & 0/9 & 0/9 & 0/9 \\
IO & 0/9 & 0/9 & 0/9 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}RT: respiratory tract.
\textsuperscript{b}IO: inner organs.

\textsuperscript{d}The frequency of re-isolation of M. gallisepticum was re-isolated/total number of birds.
Table 4
Re-isolation of *M. gallisepticum* from different locations of the respiratory tract

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Organ</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Total number of re-isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;high&lt;/sub&gt;</td>
<td>Trachea</td>
<td>2/9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6/9</td>
<td>5/9</td>
<td>13/27</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>2/9</td>
<td>4/9</td>
<td>1/9</td>
<td>7/27</td>
</tr>
<tr>
<td></td>
<td>Air sacs</td>
<td>1/9</td>
<td>3/9</td>
<td>1/9</td>
<td>5/27</td>
</tr>
<tr>
<td>R&lt;sub&gt;low&lt;/sub&gt;</td>
<td>Trachea</td>
<td>3/9</td>
<td>5/9</td>
<td>1/9</td>
<td>9/27</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>5/8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/9</td>
<td>5/9</td>
<td>19/26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Air sacs</td>
<td>4/9</td>
<td>6/9</td>
<td>8/9</td>
<td>18/27</td>
</tr>
<tr>
<td>R&lt;sub&gt;highP10&lt;/sub&gt;</td>
<td>Trachea</td>
<td>2/9</td>
<td>6/9</td>
<td>7/9</td>
<td>15/27</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>5/9</td>
<td>2/9</td>
<td>5/9</td>
<td>12/27</td>
</tr>
<tr>
<td></td>
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<td>1/27</td>
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<td>Trachea</td>
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<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
<tr>
<td></td>
<td>Air sacs</td>
<td>0/9</td>
<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of birds from which *M. gallisepticum* was re-isolated/total number of birds.

<sup>b</sup>The presence of *M. gallisepticum* in the lung sample of one bird could not be assessed due to bacterial contamination.

in the group infected with R<sub>high</sub> was obtained at day 3 from both the air sac and the lung of one bird, while it was isolated from either the trachea or the lung of three other chickens (see Tables 3 and 4). This was also valid for the group infected with R<sub>low</sub> although the total number of chickens from which *M. gallisepticum* was re-isolated (Table 3) was higher (26 out of 27) than that of the group infected with R<sub>high</sub> (17 out of 27). Interestingly, the lack of air sac lesions observed in birds infected with R<sub>high</sub> was not due to an absence of colonization of the respiratory tract, as *M. gallisepticum* was recovered from this site in several birds showing no pathological lesions. For birds of groups infected with R<sub>low</sub> and R<sub>highP10</sub>, there was a good correlation between the number of birds presenting air sac lesions (Table 1) and the number of birds from which *M. gallisepticum* was re-isolated from the respiratory tract at days 6 and 9 (Table 3). However, at day 3 these two values differed for these two groups, as not all birds that were colonized displayed air sac lesions, suggesting a delay of 3–5 days in the appearance of the pathological signs following the inoculation.

3.4. Re-isolation of *M. gallisepticum* from the inner organs

Re-isolation and identification of the mycoplasma from various inner organs was performed as described above. As illustrated in Tables 3 and 5, *M. gallisepticum* was cultivated from the inner organs of 20 out of 27 birds infected with R<sub>low</sub>. In this group, *M. gallisepticum* was recovered at least once from each organ which was assessed and which included the brain, the heart, the liver, the kidney and the spleen (Table 5). In most cases, re-isolation from the inner organs correlated with re-isolation from the respiratory tract (Table 3). For instance, *M. gallisepticum* was re-isolated from the respiratory tract and from the inner organs of 20 birds out of the 27 that composed the group infected with R<sub>low</sub>. However, in a few cases, mycoplasmas were only recovered from the respiratory tract (two birds out of nine at day 3), while the re-isolation of *M. gallisepticum* from the inner organs always correlated with the presence of mycoplasmas in the respiratory tract. This is illustrated by comparing the results of Table 3. Interestingly, while no mycoplasma was recovered from inner organs of birds infected with R<sub>high</sub>, *M. gallisepticum* R<sub>highP10</sub> was re-isolated (i) at day 6 from the kidney of one bird, and (ii) at day 9 from the heart, the kidney and the liver of a single bird, and from the liver of two birds (Table 5). Re-isolation of *M. gallisepticum* from the inner organs of birds infected with R<sub>highP10</sub> only succeeded in chickens infected for at least 6 days. In contrast, re-isolation of mycoplasmas from the inner organs of birds infected with R<sub>low</sub> was achieved in six out of nine birds already 3 days after inoculation. Overall, the results showed that in contrast to R<sub>high</sub>, R<sub>highP10</sub> (i) occasionally induced air sac lesions, (ii) was re-isolated from the heart, the kidney and the liver of four out of 27 infected birds, and (iii) induced four positive RSA reactors, two of which corresponded to those in which the avian pathogen was re-isolated from the kidney.

4. Discussion

In this report, we have formally shown the capability of *M. gallisepticum* to cross the mucosal barrier of the respiratory tract and enter the bloodstream to disseminate throughout the body by demonstrating the isolation of *M. gallisepticum* strain R from various internal organs.

Table 5
Re-isolation of *M. gallisepticum* from different inner organs

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Organ</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Total number of re-isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;high&lt;/sub&gt;</td>
<td>Heart</td>
<td>0/9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
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<tr>
<td></td>
<td>Brain</td>
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<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
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<td>Liver</td>
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<td>0/9</td>
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<td>Spleen</td>
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<td>Kidney</td>
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<td>0/9</td>
<td>0/9</td>
<td>0/27</td>
</tr>
<tr>
<td>R&lt;sub&gt;low&lt;/sub&gt;</td>
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<td>3/9</td>
<td>4/9</td>
<td>3/9</td>
<td>10/27</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>2/9</td>
<td>1/9</td>
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<td>8/27</td>
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<sup>a</sup>Number of birds from which *M. gallisepticum* was re-isolated/total number of birds.
of birds experimentally infected via aerosol. The systemic spreading which takes place within the first 3 days of infection is affected by the number of laboratory passages, since the highly passaged R\textsubscript{high} population did not induce systemic infection, while the parental low passage R\textsubscript{low} did.

Comparison of the lesion scores (Table 1) and feed conversion rates (data not shown) recorded in birds infected with R\textsubscript{high} and R\textsubscript{low} confirmed that R\textsubscript{high} is less virulent than R\textsubscript{low} as previously described by Levisohn et al.\cite{14}. Whether the inability of R\textsubscript{high} to spread from the respiratory tract to the inner organs is directly correlated with its attenuated virulence is not yet known. In the present study, birds were infected for a maximum period of only 9 days, therefore the possibility that systemic infection is delayed in birds infected with R\textsubscript{high} and would occur at a later stage cannot be ruled out. In contrast to R\textsubscript{low}, R\textsubscript{high} is not able to invade eukaryotic cells as demonstrated in vitro\cite{17}, and this difference may account for both its attenuated virulence and its inability to cause systemic infection. But again, whether cell invasiveness correlates with systemic infection and/or virulence has still to be formally demonstrated. The finding that R\textsubscript{high}P10 can induce systemic infection as well as air sac lesions in a larger number of chickens than R\textsubscript{high} may be attributed to the presence of mycoplasma cells in this population which have retained the cell invasion phenotype displayed by R\textsubscript{low}\cite{17} and supports the previous hypothesis. Recently, it was shown that R\textsubscript{high} and R\textsubscript{low} can be distinguished based on the expression of a protein designated GapA\cite{22}. This protein shares high homology with the cytadhesin P1 of \textit{M. pneumoniae} and was shown to be only expressed in R\textsubscript{low} following an irreversible mutation that had occurred in R\textsubscript{high}\cite{23}. Since adhesion is a prerequisite for cell invasion, the lack of GapA expression in R\textsubscript{high} may account for its inability to cause systemic infection, and to induce air sac lesions by preventing efficient binding of mycoplasmas to the mucosa. In vitro, R\textsubscript{high} was shown to bind to HeLa and CEF\cite{17}; however, the situation with tracheal epithelial cells along with the in vivo setting might be different. Comparison of the protein profile of the three passages derived from strain R revealed the expression of additional proteins in R\textsubscript{high}P10, notably of two prominent products of approximately 31 and 46 kDa not previously detected in R\textsubscript{high}, or in R\textsubscript{low}\cite{17}. These additional proteins have yet to be characterized and might be responsible for the differences in translocation, virulence and/or cell invasion observed among the three mycoplasma populations derived from strain R.

The absence of RSA-positive reactors in birds infected with R\textsubscript{high} for a period of 9 days indicates their lack of circulating antibodies and contrasts with the situation observed with groups infected with R\textsubscript{low} or R\textsubscript{high}P10. However, this does not rule out the presence of local immunity which might be, as postulated\cite{24,25}, more important than circulating antibodies in providing protection from \textit{M. gallisepticum} disease. So far, control strategies developed to prevent and eradicate \textit{M. gallisepticum} infections have only been partially efficient. The capacity of \textit{M. gallisepticum} to hide from host defenses by entering eukaryotic cells and to navigate through the body may participate in its survival and in the persistence of infection. The identification of the components that are responsible for the differences observed between R\textsubscript{low} and R\textsubscript{high} emerges as a crucial step to design more appropriate control strategies and to understand the virulence mechanisms promoting \textit{M. gallisepticum}-associated diseases.

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