Counterimmunoelectrophoresis for Diagnosis of Infectious Bursal Disease: Comparative Efficacy of Three Different Electrophoresis Buffers

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Primary Audience: Veterinarians, Poultry Veterinarians, Poultry Scientists, Microbiologists

SUMMARY

The bursas of Fabricius of 48 broiler chickens (meat type) of age groups 3 to 7 wk, affected with infectious bursal disease (IBD) were tested by counterimmunoelectrophoresis (CIE) by using 3 different buffers, namely, barbital buffer, Tris-glacial acetic acid-EDTA (TAE) buffer, and Tris-boric acid-EDTA buffer. Eighty-five percent of the samples were positive when the TAE buffer was used, whereas the Tris-boric acid-EDTA and barbital buffers yielded 81% positive test results. The barbital buffer, which is commonly used in CIE for diagnosis of IBD, is not readily available and is considered carcinogenic. The TAE buffer was found to be safe and was a superior buffer for diagnosing IBD in a CIE test.

Key words: infectious bursal disease, diagnosis, counterimmunoelectrophoresis, counterimmunoelectrophoresis buffer

DESCRIPTION OF PROBLEM

Infectious bursal disease (IBD) is a contagious immunosuppressive disease of chickens caused by a birnavirus [1, 2]. Clinical disease and mortality occur in young chickens, but often the infection is essentially subclinical in the early age groups and in relatively older birds when the bursa is not fully developed or is atrophied. The IBD virus primarily affects the bursa of Fabricius. Inflammation and swelling of the bursa of Fabricius is almost a pathognomonic lesion, but bursal atrophy has been recorded from the later stage of infection. Escherichia coli infection also causes swelling of the bursa of Fabricius [3]. Other infectious agents and noninfectious conditions, such as dehydration, can also produce gross lesions in the bursa that resemble IBD. The bursa of Fabricius is the main organ of B-lymphocyte production and these B lymphocytes are responsible for the production of antibody or humoral immunity. Bursal damage leads to immunosuppression. Thus, accurate and rapid diagnosis of the disease is important to implement effective control

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measures. Serological tests such as agar gel immunodiffusion (AGID) [4, 5], counterimmunoelectrophoresis (CIE) [6], immunofluorescence [7], immunoperoxidase [8], and others have been used for the demonstration of viral-specific antigen in bursal tissues. Among these serological tests, the CIE test is less expensive, more reliable, quick, and easy to adopt. For CIE, a barbital buffer is commonly used as the electrophoresis buffer. The barbital buffer is known to be carcinogenic [9] and its use has been restricted.

In the current study, an attempt was made to compare the efficacy of different buffers in CIE for diagnosing IBD in chickens.

MATERIALS AND METHODS

IBD Antigen

Forty-eight tissue samples of the bursa of Fabricius were collected from 21 different natural outbreaks in broiler birds (meat type) from 3 to 7 wk of age. Antigens were prepared as described earlier [6]. Individual tissues of the bursa of Fabricius were homogenized, and a 50% suspension (wt/vol) was made in PBS. The homogenate was frozen and thawed 3 times and clarified by centrifugation at 1,207 \( \times \) g for 10 min. The supernatant was then used as the test antigen. Similarly, bursas of Fabricius were collected and processed from 10 healthy unexposed, unvaccinated, separately maintained 4-wk-old chickens and used as negative control antigen for the test. Positive control antigen of the bursa of Fabricius, prepared earlier in this laboratory by infecting susceptible healthy chickens with classical serotype-1 IBD virus, was also used.

IBD Virus Antiserum

Antiserum was produced as described earlier [4]. Three-week-old specific serum antibody-negative White Leghorn chicks were inoculated with 100 chick infective dose 50 of IBD virus classical serotype-1. Blood was collected 21 d after infection from the surviving birds and serum was separated. Antiserum had a quantitative CIE titer of 5 log2 against the IBD virus.

CIE

Three different buffers were used for the CIE test. In the first portion of the experiment, 4 mL of molten 1% agarose gel of high electroendosmosis (EEO) in 0.06 M barbital (sodium barbital) buffer (pH 8.6) [10] was layered on microscopic glass slides (75 m \( \times \) 25 mm). Pairs of wells of 5-mm diameter and 2-mm interspace were cut in such a way that each slide contained 2 rows of 3 pairs of wells [11]. The same 0.06 M barbital buffer was used as the electrophoresis buffer in the buffer tank. Infectious bursal disease antigen was added to the cathode side and IBD antiserum was added to the anode side. Known IBD-positive control antigen and known IBD-negative control antigen were added to each slide. The CIE test was performed with a constant current of 10 mA per slide for 45 min. The slides were then dipped in an 8% sodium chloride solution for 30 min and the results were read under diffuse light.

In the second portion of the experiment, a 1% agarose (high-EEO) gel was prepared in Tris-glacial acetic acid-EDTA (TAE; i.e., 0.04 M Tris acetate and 0.001 M EDTA) buffer (pH 8.0). The same TAE was used as the electrophoresis buffer, whereas other conditions were the same as described above.

In the third portion of the experiment, a 1% agarose (high-EEO) gel was prepared in Tris-boric acid-EDTA (TBE; i.e., 0.045 M Tris borate and 0.001 M EDTA) buffer (pH 8.0). The same TBE buffer was used as the electrophoresis buffer; other conditions were the same as described in the first experiment. The TAE and TBE buffers were prepared as described previously [12].

AGID Test

The AGID test was performed with microscopic slides (75 \( \times \) 25 mm) layered with 4 mL of 1% agarose (low-EEO) gel containing 8% sodium chloride [13]. Wells of 4-mm diameter and 2-mm interspace were cut. Antiserum was added to the central wells and antigen was added to the peripheral wells. Known IBD-positive antigen and known IBD-negative antigen were also added as controls. Slides were kept at room temperature in a humidified box and read at 24, 48, and 72 h under diffused light.
Table 1. Comparison of the results of 3 different electrophoresis buffers to demonstrate infectious bursal disease virus antigen in bursa of Fabricus tissues of chickens by counterimmunoelectrophoresis

<table>
<thead>
<tr>
<th>Total samples</th>
<th>Buffer</th>
<th>Positive (n)</th>
<th>Negative (n)</th>
<th>Positive (%)</th>
<th>Test agreement (%)</th>
<th>Kappa statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>Barbital</td>
<td>39</td>
<td>9</td>
<td>81</td>
<td>95</td>
<td>0.97</td>
</tr>
<tr>
<td>48</td>
<td>TAE</td>
<td>41</td>
<td>7</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>Barbital</td>
<td>39</td>
<td>9</td>
<td>81</td>
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<tr>
<td>48</td>
<td>TBE</td>
<td>39</td>
<td>9</td>
<td>81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*TAE = Tris-glacial acetic acid-EDTA; TBE = Tris-boric acid-EDTA.

The test results were analyzed for the percentage of agreement by using kappa statistics [14]. The kappa statistic is a decimal measurement of agreement between 2 tests, especially in the absence of a standard, and is defined as

\[ k = \frac{(a + d - p)}{(1 - p)} \]

where \( p \) is the probability, \( a \) is the number of samples positive by both tests, \( b \) is the number of samples negative with the barbital buffer but positive with the TAE or TBE buffer, \( c \) is the number of samples positive with the barbital buffer but negative with the TAE and TBE buffers, and \( d \) is the number of samples negative by all the tests. When the \( k \) value is above 0.81, the test of agreement is almost perfect. Sensitivity, specificity, and accuracy were calculated with the formulas \( a/(a + b) \), \( b/(c + d) \), and \((a + d)/(a + b + c + d)\), respectively.

RESULTS AND DISCUSSION

In the AGID test, IBD virus antigen was demonstrated in 18 (37.5%) bursal tissues. Ten known negative samples were also negative, whereas the positive control antigen was always positive. Results obtained by CIE with the 3 different buffers are listed in Table 1. Positive samples were demonstrated by a line of precipitation, whereas negative samples did not show any line of precipitation (Figure 1). The percentage of agreement in CIE between tests using the barbital buffer and those using the TAE buffer was 92%, and between the barbital buffer and the TBE buffer the percentage of agreement was 95%. The kappa statistics indicated perfect agreement in both cases. In the CIE test, 81, 85, and 81% of samples were positive for IBD

Table 2. Sensitivity, specificity, and accuracy\(^1\) assessed when using 3 different buffers in counterimmunoelectrophoresis to diagnose infectious bursal disease virus antigen\(^2\)

<table>
<thead>
<tr>
<th>Cell</th>
<th>Barbital</th>
<th>TAE</th>
<th>Number</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
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<tbody>
<tr>
<td>a</td>
<td>+</td>
<td>+</td>
<td>38</td>
<td>0.93</td>
<td>0.86</td>
<td>0.92</td>
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<tr>
<td>b</td>
<td>−</td>
<td>+</td>
<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>+</td>
<td>−</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>−</td>
<td>−</td>
<td>6</td>
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<td></td>
</tr>
<tr>
<td>a</td>
<td>+</td>
<td>+</td>
<td>38</td>
<td>0.97</td>
<td>0.89</td>
<td>0.96</td>
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<tr>
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<td>−</td>
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<tr>
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<tr>
<td>d</td>
<td>−</td>
<td>−</td>
<td>8</td>
<td></td>
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<td></td>
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</tbody>
</table>

\(^1\)Sensitivity = \(a/(a + b)\); specificity = \(b/(c + d)\); and accuracy = \((a + d)/(a + b + c + d)\).

\(^2\)Test of agreement = \(a + d/n; k = (a + d - p)/(1 - p); p = (a + b)(a + c) + (c + d)(b + d)\), where \( a \) is the number of samples positive by both tests, \( b \) is the number of samples negative with the barbital buffer but positive with the Tris-glacial acetic acid-EDTA (TAE) or Tris-boric acid-EDTA (TBE) buffer, \( c \) is the number of samples positive with the barbital buffer but negative with the TAE and TBE buffers, and \( d \) is the number of samples negative by all the tests.
Figure 1. Picture showing the line of precipitation between wells of infectious bursal disease (IBD) virus-positive bursa suspension and antiserum. Well 1 was filled with antiserum, well 2 was filled with test sample (IBD virus-infected bursa), well 3 was filled with positive control bursa (IBD virus-infected bursa), and well 4 was filled with negative control bursa (uninfected).

virus when using the barbital, TAE, and TBE buffer, respectively. Table 2 shows the sensitivity, specificity, and accuracy of CIE when using the TAE and TBE buffers with the barbital buffer as the standard. When TAE was used as a buffer, the line of precipitation was observed immediately in many samples, but when the other 2 buffers were used, the line of precipitation was clearly seen in most cases after dipping the slides for approximately 30 min in an 8% sodium chloride solution. No such line of precipitation was observed with the 10 samples collected from healthy unvaccinated chickens. The CIE results of all 10 negative samples, collected from the unvaccinated healthy chickens, were negative with all 3 of the buffers tested.

Infectious bursal disease is easily recognizable by gross pathological lesions of the bursa of Fabricius. Swelling with edema of the bursa of Fabricius is almost a characteristic feature. However, such lesions are rare in acute [6] and subacute cases. Escherichia coli infection also produces similar kinds of lesions in the bursa of Fabricus [3]. For a reliable and simple diagnosis, the AGID test is being used in most field-level laboratories, where facilities are limited. But the major disadvantages of the AGID test are that it is less sensitive and takes a longer time (approximately 24 to 72 h). Molecular tests such as PCR are expensive and are not affordable by small field-level laboratories. Counterimmunoelectrophoresis was found to be more sensitive than AGID in the diagnosis of IBD [6], and the results of the test could be obtained in approximately 45 min. The barbital buffer is commonly used in CIE tests for diagnosing IBD. The barbital buffer is known to be carcinogenic [9] and is not commonly available in the market. In the current study, TAE was found to be a superior buffer for CIE in diagnosing IBD. The line of precipitation was readily observed in most of the positive samples when the TAE buffer was used, but when other buffers were used, dipping the gels in an 8% sodium chloride was often required to demonstrate a clear line of precipitation. The kappa values showed almost perfect agreement between CIE tests when using the barbital buffer as the standard with the TAE and TBE buffers. The sensitivity, specificity, and accuracy of CIE were quite good when using the TAE and TBE buffers (Table 2). Although the barbital buffer is commonly used, because of its demerits and limited availability, the TAE buffer can be used as an alternative; it was found to be superior even to the barbital buffer in CIE for the diagnosis of IBD.

CONCLUSIONS AND APPLICATIONS

1. The TAE buffer was found to be safe and was a superior buffer compared with the barbital and TBE buffers for diagnosing IBD when using CIE.
REFERENCES AND NOTES


10. Sigma Chemical Co., St. Louis, MO.


Acknowledgments

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