Diagnostic specificity of ELISA-based tests for the detection of antibodies to Rift Valley Fever virus in French ruminants

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SUMMARY

The Rift Valley Fever (RVF) is a zoonotic, mosquito-transmitted viral disease present in Africa and in the Arabian Peninsula. For asserting the absence of the RVF virus in temperate countries of Europe and America, the evaluation of the diagnostic performances of the existing serological assays is required. The aim of this study was to assess the diagnostic specificity of commercially available ELISA kits for the detection of the early IgM and delayed IgG antibodies against RVF virus in sheep, goats and cattle from France. Among the 2,154 ruminant sera tested by IgM-capture ELISA, 2 sheep and 3 goat sera were above the recommended cut-off value. For the IgG-sandwich ELISA, 26 sheep sera but none goat or cattle sera out of the 2,506 ruminant samples tested gave positive results. As they gave negative results with a competitive ELISA and/or virus neutralisation test, they were considered as false positive. These results confirm the high diagnostic specificity in French ruminants of commercially available IgG-sandwich ELISA (97.3% in sheep, 100% in goats and cattle) and IgM-capture ELISA (99.54% in goats, 99.75% in sheep and 100% in cattle), which are particularly helpful for the disease surveillance programs in RVF-free countries.

Keywords: Rift Valley Fever, serology, ELISA, IgG, IgM, specificity, ruminants.

Introduction

The Rift Valley fever (RVF) virus is a mosquito-borne pathogen with high potential to cause explosive outbreaks of severe human and livestock diseases. Although historically limited to Africa and Madagascar, more recently severe outbreaks of the disease were recorded in 2000 on the Arabian Peninsula and in 2008 on the Archipelago of Comoros, including the French department of Mayotte [17]. The RVF virus (RVFV) is the prototypical Phlebovirus of the family Bunyaviridae with a lipid-membrane envelope and surface protrusions of two viral glycoproteins [16], containing a tripartite, single stranded, negative-sense RNA genome [6]. The majority of infections with RVFV in humans are characterized by a mild self-limiting febrile illness but in a small proportion of cases more severe complications, including hepatitis, delayed onset neurological disease, retinitis, or a hemorrhagic syndrome with high mortality occur. In sheep, goats and cattle, RVF epizootics usually manifests as “abortion storms” and high mortality rates among new-born animals [1]. The virus is transmitted to animals mostly by bites of vector competent mosquitoes of many genera (Aedes, Anopheles, Culex, Eretmapodites, and Mansonia) during seasons of high rainfall.

The RVF diagnosis relies on specific-antibody detection, virus isolation and genome amplification [8]. Among serological tests, ELISA tests using gamma-irradiated antigens tend to replace classical tests such as virus neutralization [8] and haemagglutination - inhibition tests [19] which require manipulation of live virus and thus their use is restricted to BSL-3 or BSL-4 facilities.

RéSUMÉ

Spécificité des tests ELISA pour la détection des anticorps dirigés contre le virus de la Fièvre de la vallée du Rift chez des ruminants français

La fièvre de la vallée du Rift (FVR) est une arbovirose et une zoonose présente sur le continent africain et dans la péninsule arabe. Afin de certifier l’absence de ce virus dans les régions tempérées d’Europe et d’Amérique, l’évaluation des performances diagnostiques des tests sérologiques existants est indispensable. L’objectif de cette étude a donc été de déterminer la spécificité des trousses ELISA disponibles pour la détection des anticorps dirigés contre le virus de la FVR de classes IgM (anticorps précoces) et IgG (anticorps plus tardifs) dans le cheptel français de ruminants (moutons, chèvres et bovins). Parmi les 2 154 sérumes testés pour la recherche d’IgM dirigées contre ce virus, seulement 5 prélèvements (2 de moutons et 3 de chèvres) étaient au-dessus du seuil de positivité. En ce qui concerne les IgG, 26 sérumes de moutons mais aucun de chèvre ou de bovin parmi les 2 506 échantillons testés se sont avérés positifs. Néanmoins, la réalisation d’un test ELISA par compétition et/ou la technique de séro-neutralisation virale ont confirmé le caractère faussement positif de ces sérumes. Au final, la forte spécificité de ces 2 tests sérologiques ELISA a été confirmée dans le cheptel français malgré de légères différences entre les espèces (97.3 % chez les moutons, 100 % chez les chèvres et les bovins pour les IgG, 99.54 % chez les chèvres, 99.75 % chez les moutons et 100 % chez les bovins pour les IgM) et conforte leur intérêt dans la surveillance de la maladie dans les pays indemnes de RVF.

Mots clés : Fièvre de la vallée du Rift, sérologie, ELISA, IgG, IgM, spécificité, ruminants.
Recent risk assessment studies performed by national or European agencies have re-evaluated the risk of introduction of the RVF virus in Europe [4, 7, 15] or in other disease free countries such as the USA [2, 10]. Among recommendations proposed by these studies, there is a need of evaluating the performances of existing diagnostic tools under European conditions since their diagnostic characteristics are of concern for the decision-makers in the context of clinical diagnosis or quantitative risk assessment as well as for the epidemiologists [9]. Such studies have been already performed in African ruminants particularly by producers of ELISA kits [12] and recently for the competitive ELISA in French ruminants [3]. The aim of the present study was to evaluate the specificity of the commercially available IgG-sandwich ELISA and IgM-capture ELISA for the detection of IgM and IgG antibodies [12]. Sera collected from French sheep, goats and cattle were assayed according to the guidelines recommended for the validation of veterinary diagnostic tests [9]. As France is officially free of RVF, all these sera should be regarded as negative for specific antibodies. The diagnostic specificity estimates are important particularly for surveillance studies and for laboratory testing of RVF suspected cases.

Materials and Methods

FIELD SERA

A total of 2,506 ruminant sera were tested in IgG-sandwich ELISA and 2,154 sera in IgM-capture ELISA (Table I). Sera were collected from various farms from the department of Rhône, France during the 2008 campaign of official prophylaxis for brucellosis. These sera were kindly provided by Dr J. Vialard from the Department Veterinary Laboratory of Rhône (VetAgro Sup, Campus Vétérinaire de Lyon, Marcy-l’Etoile, France). As France is officially free of RVF, collected sera have been considered as negative for RVF antibodies.

VIRUS NEUTRALIZATION TEST

The virus neutralization test (VNT) was conducted according to a method described previously [8]. This test was performed in the BSL-3 facility of the Pasteur Institute in Paris (Génétique moléculaire des Bunyavirus, Pasteur Institute, Paris, France) or in the BSL-3 facility of the Afssa-Lyon (Afssa site de Lyon, Lyon, France).

ELISA TESTS

The ELISA kits (Biological Diagnostic Supplies Limited (BDSL), Scotland, UK) were used according to the manufacturer protocols and published procedures. These kits have been originally developed by the Special Pathogens Unit of the National Institute for Communicable Diseases (SPU-NICD), Sandringham, South Africa [11, 12, 14]. Recommended internal quality controls (IQC) limits for a set of controls were strictly applied during the study. When one of the internal controls did not fall within the IQC limits on a particular ELISA plate, sera were re-tested and only results obtained during the validated run were taken into account for the final analysis. Three different batches of kits were used in this study. Flow charts of the IgG-sandwich and IgM-capture ELISAs are shown in Figures 1 and 2. The cut-off values expressed as percentage positivity (PP) of a high-positive control serum used in this study were the same as specified by the kit producer: for IgG, 11.1% [sheep], 18.1% [goat] and 16.4% [bovine] and for IgM, 8% [sheep], 9.5% [goat] and 14.3% [bovine]. Sera found positive for specific IgG antibody were also tested with the virus neutralization test and in an inhibition ELISA, an assay also available from the BDSL and originally developed and validated by the SPU-NICD in South Africa [13]. For each microplate, strong (C++) and low (C+) positive sera and negative serum (C-) were included as recommended by the manufacturer. In addition, one IgG and one IgM positive sheep serum kindly provided by Dr T. GERDÈS (OVI, Onderstepoort, South Africa), were respectively added on each microplate for IgG and IgM ELISA tests.

Results

Concerning the use of the commercial ELISA kits, there were variations of the optical density (OD) readings for internal controls between the different batches of kits used in this study. These variations between batches were circumvented by applying strictly the protocol defined by the manufacturer.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Species</th>
<th>Total</th>
<th>Negative results (%)</th>
<th>Positive results</th>
<th>Specificity (%)</th>
<th>Confidence interval (%)</th>
</tr>
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<tbody>
<tr>
<td>IgG</td>
<td>Sheep</td>
<td>967</td>
<td>941</td>
<td>26</td>
<td>97.31</td>
<td>96.03 - 98.20</td>
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<tr>
<td></td>
<td>Goat</td>
<td>664</td>
<td>664</td>
<td>0</td>
<td>100.00</td>
<td>99.28 - 100.00</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>875</td>
<td>875</td>
<td>0</td>
<td>100.00</td>
<td>99.45 - 100.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>2,480</td>
<td>26</td>
<td>98.96</td>
<td>98.46 - 99.30</td>
</tr>
<tr>
<td>IgM</td>
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<td>793</td>
<td>791</td>
<td>2</td>
<td>99.75</td>
<td>99.29 - 99.96</td>
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<tr>
<td></td>
<td>Goat</td>
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<td>656</td>
<td>3</td>
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<tr>
<td></td>
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<td>702</td>
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<td>100.00</td>
<td>99.32 - 100.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>2,149</td>
<td>5</td>
<td>99.77</td>
<td>99.42 - 99.91</td>
</tr>
</tbody>
</table>

Table I: Specificity of the commercial ELISA kits for the detection of IgG or IgM antibodies to Rift Valley fever virus in domestic ruminants.
Results obtained with the ruminant sera tested by the IgM or IgG ELISA are given in Table I. The overall specificities for the IgG and IgM ELISA kits were 98.96% and 99.77% respectively. Considering the 3 ruminant species (sheep, goat and cattle), the sheep sera had the lowest diagnostic specificity with the IgG ELISA kit (97.31%) whereas bovine sera exhibited 100% specificity in both IgM and IgG ELISA kits. In goats, the IgG ELISA kit specificity was maximal (100%) while 3 sera gave a positive reaction with the IgM ELISA kit (specificity: 99.54%).

Most of the sera found positive were just above the cut-off value defined by the producer of kits but a few sera had high or very high PP values. For example, among the 26 sheep sera tested positive for anti RFV virus IgG, 12 had PP values comprised between 11.1 PP (equivalent to the cut-off PP value) and 20.0. When the 26 sheep sera were re-tested in the inhibition ELISA, they gave negative results except for 3 sera. Interestingly, all of them also gave positive results in an in-house ELISA kit made by Pasteur Institute (data not shown) but all of the 26 IgG-sandwich positive sera were negative in the virus neutralization test. By contrast, a positive reaction for the anti RFV virus IgM was encountered in only 2 sheep sera (specificity: 99.75%, Table I). However, these 2 IgM-positive sera did not belong to the 26 IgG-positive sera.

Discussion

The estimates for diagnostic specificity of RVF ELISA kits obtained in this study are very close to those initially published by the kit designers: 99.10%, 99.90% and 99.67% for sheep, goats and bovine sera, respectively [12]. In this study, the IgG-sandwich ELISA gave a higher number of false-positive results in sheep sera (26 of 967); these positive sera could be regarded as false-positive as the virus neutralization test and/or the inhibition ELISA gave negative results, confirming high diagnostic specificity of these assays [3, 13]. There is no clear answer how to explain false-positive results obtained in the IgG-sandwich ELISA. A potential cross-reactivity with other Bunyaviruses may be reasonably excluded as these viruses, and particularly those of the Phlebovirus genus have never been identified to date in European domestic ruminants and RVF virus does not share any known cross-reactivity with other Phlebovirus [18], except for viruses present only in Latin America [21]. An explanation could be the presence of a “sticky” factor present in sera of some animals in relation, for example, with the food as 5 clusters (ranging from 2 animals to 4 animals per cluster) of false-positive sheep were identified among the 26 positive sera and belonging to the same farm.

Results of the study confirm that the currently available ELISAs can be used as reliable diagnostic tools in disease surveillance and control programs. However, these tests are based on inactivated whole virus as antigen, and it is expected that in a near future, they will be replaced by ELISA tests utilizing recombinant antigens, providing these assays are shown to have comparable or higher diagnostic accuracy. An ELISA based on a recombinant antigen has a number of important advantages, including safety for laboratory workers and cost-effectiveness format [5, 20].

Acknowledgement

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References


