Canine leishmaniasis in Algeria: True prevalence and diagnostic test characteristics in groups of dogs of different functional type

Amel Adela, Claude Saegerman, Niko Speybroeck, Nicolas Praet, Bjorn Victor, Redgi De Dekken, Abdelkrim Soukela, Dirk Berkvens

A Bayesian approach was used to assess the prevalence of Canine leishmaniasis and evaluate three serological diagnostic tests: indirect fluorescent antibody test (IFAT), direct agglutination test, and particle gel immuno-assay (PaGIA) for Canine leishmaniasis (CL) in Algiers. Four hundred and sixty-two dogs were involved in this study and divided in four groups according to their functional type: stray dogs, farm dogs, national guard dogs and pet dogs. The stray dog group showed the highest prevalence of leishmaniasis (11.7%), followed by the national guard dogs (9.7%) and the farm dogs (5.9%). IFAT was shown to be the most sensitive test in all groups. However, IFAT specificity was considerably lowered in the farm dog group: 65.2% versus 94.5% for the stray dogs. A considerable drop in PaGIA specificity was noted in the stray dogs group. The results of the current study demonstrate the variability of test characteristics in different situations and underline the danger of using standard values, without verifying their appropriateness for the specific purposes.

1. Introduction

Leishmaniasis are visceral and cutaneous parasitic diseases, found worldwide (Dereure, 1993). Visceral human leishmaniasis (VHL) – also known as kala azar – is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. If left untreated, the fatality rate in developing countries can be as high as 100% within 2 years (WHO, 2009). Leishmania infantum, an intracellular protozoan, is the causal agent of the human and animal visceral leishmaniasis in the Mediterranean basin (Sideris et al., 1999). It is transmitted by the bites of female sandflies of the genus Phlebotomus (Phlebotominae, Diptera) (Killick-Kendrick, 1990), such as Phlebotomus perniciosus in Algeria (Izri et al., 1990).

Leishmaniasis is a zoonotic disease for which dogs are considered to be the chief reservoir of the parasite (Solano-Gallego et al., 2001) and are considered responsible for the persistence of VHL (Abranches et al., 1991). Currently, the annual worldwide incidence of VHL is estimated at around 500,000 cases (Desjeux, 2004). However, the World Health Organisation estimates that only a third of the new cases is officially declared.

In Algeria, VHL has been known since long to occur in the humid and sub-humid areas (Dereure, 1993). The annual number of human cases is estimated at about 400 (Benikhlef et al., 2001), with an incidence rate of 0.41 per 100,000 inhabitants. By Wilaya (Province), this incidence rate varies between 0.11 and 2.85 (Benhabyles and Boughoufal, 2004). Harrat et al. (1995) reported an...
unmistakable spread of the disease during the preceding period to areas which were until then uninfected, giving rise to cases of VHL in children who had never left the capital. These particular cases were explained by a recrudescence of *Canine leishmaniasis* (CL) in the Wilaya of Algiers.

At the Institut Pasteur d’Alger, 37% of 1800 dogs presenting a strong suspicion or a risk of infection tested positive in an immunofluorescence antibody test (IFAT) (Harat and Belkaid, 2003).

CL is a severe, often fatal systemic disease (Ferrer et al., 1995). The clinical signs are variable and can mimic other infections (Office, 2005). More than half of the infected animals remain asymptomatic during a variable period of incubation that can range from three months to several years (Cardoso et al., 2004; Ferrer et al., 1995). They can, however, be as infective to the vector as symptomatic dogs (Molina et al., 1994). For these reasons, it is important to diagnose CL as early as possible to prevent further transmission (Schallig et al., 2002).

The direct parasitological test suffers from low sensitivity (El Harith et al., 1989) and the detection of specific anti- *Leishmania* antibodies in canine sera remains an important diagnostic tool (Schallig et al., 2002; Mettler et al., 2005). However, no gold standard test exists for either VHL or for CL (Boelaert et al., 1999a,b). Simultaneous estimation of prevalence of infection and diagnostic test characteristics has been carried out successfully when applying several diagnostic tests to every individual, using a Bayesian approach combining test results and external information, such as experimental evidence and expert opinion (Adel, 2002; Berkvens et al., 2006; Geurden et al., 2006; Lesaffre et al., 2007). This Bayesian modelling allows resolving issues related to the lack of a gold standard for *L. infantum* infection in the interpretation of serological survey data: prevalence and test characteristics were previously often incorrectly determined against parasitology, known to be poorly sensitive, as a reference test (Boelaert et al., 1999a; Dye and Vidor, 1993).

A survey, involving 462 dogs, was conducted between 2004 and 2005 in the area of Algiers. The purpose of the study was to compare three serological diagnostic tests and to assess the prevalence of the CL in this area using a multitesting Bayesian model. This is the first Bayesian model to allow a comprehensive understanding of major differences in prevalence and test characteristics in function of the functional use of dogs for CL in Algeria.

2. Materials and methods

2.1. Animals

A random cross-sectional survey was carried out in the canine population of the Algiers area. No stratification was made according to breed, sex or age of the animal. Thus, 462 sera were randomly collected between late October 2004 and the end of June 2005. The samples were accompanied by an epidemiological questionnaire detailing the origin of the animal, its age, sex and breed, the environment in which it was kept and the eventual presence of clinical symptoms.

Four groups were defined in function of the functional use of the animal: stray dogs (*n* = 218), farm dogs (*n* = 87), national guard (Gendarmerie) dogs (*n* = 92) and pet dogs (*n* = 65).

2.2. Serology

Blood was collected from the radial vein in dry tubes. Three diagnostic tests were used: indirect fluorescent antibody test (IFAT), direct agglutination test (DAT) and a particle gel immuno-assay test (Vet-Pagiatest, PaGIA).

IFAT was performed as described by Vercammen and De Deken (1996) and Mancianti and Meciani (1988), using promastigotes of *L. infantum* as the antigen. Anti- *Leishmania* antibodies were detected using rabbit anti-dog Immunoglobulin G (IgG) secondary antibodies (whole molecule) conjugated to fluorescein isothiocyanate (FITC) (Sigma–Aldrich, St Louis, MO, USA). A cut-off dilution of 1:128 was used (Abranches et al., 1983, 1991). It should be noted that this cut-off value is different from the one traditionally applied in Algeria, which is 1:80.

DAT was performed with the commercial kit supplied by the Institute of Tropical Medicine (Antwerp), using a cut-off value of 1:320 (El Harith et al., 1989; Boelaert et al., 1999a). The DAT/CL antigen is a freeze-dried suspension of trypsin-treated, fixed and stained culture from promastigotes of *L. infantum*. In the presence of antibodies to *Leishmania* (IgG), the DAT/CL antigen is agglutinated.

PaGIA is a commercial gel-based rapid antibody test (DiaMed AG, Cressier sur Morat, Switzerland) that was carried out according to the instructions of the supplier (Mettler et al., 2005).

The test is based on the rK39, a recombinant protein with a repetitive epitope closely conserved among members of the *Leishmania donovani* complex (Reed, 1995).

2.3. Statistical analysis

The interpretation of results of diagnostic tests require the inclusion of the so-called external information: the data at hand (test results) do not contain any information about test sensitivity and test specificity. Inclusion of this information is essentially a subjective process. For example, the person interpreting the test results may decide to use the values of test sensitivity and test specificity as supplied by the producer of the testkit. This is a personal decision, not linked to or influenced by the data. Combining prior (external) information with data (using a likelihood function) is the domain of Bayesian statistics.

The challenge in this case is to examine whether or not the prior information is in accordance with the data. Test sensitivity and/or specificity may have been determined under conditions not compatible with a specific application of the test. For example, the test characteristics were determined in a temperate region using temperate breeds and the test is applied to tropical breeds in a tropical setting. Using the criteria outlined further it is possible to detect such a contradiction. As explained by Lesaffre et al. (2007) absence of proof is also here no proof of absence: not finding a contradiction between the data and the prior information does not guarantee that the final model yields the best possible estimates of test characteristics and true prevalence, only that there is no evidence of
lack of accordance between prior (external, expert) information/opinion and data and that the estimates are the best conditional on the data and the external information.

A multi-testing model (several diagnostic tests applied to each individual, in this case three tests) results in data that can be represented by a so-called multinomial model (Olkin et al., 1994). A multinomial model, based on conditional probabilities as developed by Berkvens et al. (2006), was adapted to the Bayesian approach using the results of the three tests. Prior information on the test characteristics was extracted from various publications.

- prevalence constrained to [0–0.5] (Harrat and Belkaid, 2003; Boelaert et al., 1999a; Natami et al., 2000; Bettini and Gradoni, 1986),
- sensitivity and specificity of IFAT both constrained to [0.9–1] (Boelaert et al., 1999a),
- sensitivity of DAT constrained to [0.5–1] (Boelaert et al., 1999a),
- specificity of DAT constrained to [0.4–1] (Harrat, 2006).

The analysis was carried out in WinBUGS 1.4 (Spiegelhalter et al., 2003) and R (R Foundation and Statistical Computing, 2008). Criteria for model fit were evaluated as proposed previously. Briefly, the Bayesian p-value (Bayes-p, posterior predictive check) (Gelman et al., 2004) detects lack-of-fit of the model and the data. The Deviance Information Criterion (DIC) (Spiegelhalter et al., 2002) ensures that the most parsimonious model is used and \( pD \) (Spiegelhalter et al., 2002) represents the number of parameters effectively estimated by the model. These three criteria were used to assess fit of prior information and data. The latter two statistics were evaluated at the posterior means of the multinomial probabilities and the posterior means of the parent nodes as suggested by Berkvens et al. (2006). All models were run using three chains, a burn-in period of 5000 iterations and another 5000 iterations to obtain estimates (posterior distributions).

As there were multiple opinions of experts, the priors can take various values. The lower limit of the prior interval was first defined as the minimal value for that variable obtained from the literature and the expert opinions. Then, by examining the behaviour of DIC, \( pD \) and Bayes-p, the constraints were adjusted (dropped or relaxed) as indicated by the criteria outlined above and the visual appraisal of the posterior density distributions of the parent nodes and calculated variables.

### 3. Results

The results of applying the three diagnostic tests to the 462 sera are shown in Table 1.

Assuming that IFAT is a gold standard (Boelaert et al., 1999a), the true prevalence and the test characteristics for the two other tests can be computed from Table 1. The results are shown in Table 2.

The Bayesian analysis showed that the prior constraints outlined in Section 2 could be applied without any adaptation to the stray dog group. These priors were however not in agreement with the results obtained for the farm dogs and the national guard dogs.

For the farm dogs it was necessary to decrease the lower limit for IFAT specificity to 0.60, i.e. using the domain [0.6–1]. The specificity of DAT could be constrained to the domain [0.8–1], thereby approaching the findings of Boelaert et al. (1999a). An extra prior constraint of [0.8–1] was applied for PaGIA specificity (Mettler et al., 2005).

For the national guard dogs it was also necessary to reduce the lower limit for the IFAT sensitivity prior domain to 0.8, i.e. using a uniform distribution over the domain [0.8–1].

The result of the Bayesian analysis is shown in Table 3.

The stray dog group showed the highest prevalence (11.7%), followed by the national guard dogs (9.7%) and the

### Table 1

Serological test results: number of results per test result combination, all groups combined and per individual group.

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>All groups</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFAT</td>
<td>DAT</td>
<td>PaGIA</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>254</td>
<td>462</td>
</tr>
</tbody>
</table>

Number: 462 sera are shown in Table 1.

### Table 2

Prevalence by group and sensitivities and specificities of DAT and PaGIA test, given IFAT as a gold standard.

<table>
<thead>
<tr>
<th>Group</th>
<th>Positives (%)</th>
<th>DAT</th>
<th>PaGIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Se (%)</td>
<td>Sp (%)</td>
<td>Se (%)</td>
</tr>
<tr>
<td>Stray</td>
<td>15.6</td>
<td>44.1</td>
<td>94.6</td>
</tr>
<tr>
<td>Farm</td>
<td>40.2</td>
<td>11.4</td>
<td>98.1</td>
</tr>
<tr>
<td>Guard</td>
<td>17.4</td>
<td>25.0</td>
<td>84.2</td>
</tr>
<tr>
<td>Pet</td>
<td>18.5</td>
<td>33.3</td>
<td>98.1</td>
</tr>
</tbody>
</table>

Stray: stray dogs \( n = 218 \); farm: farm dogs \( n = 87 \); guard: national guard dogs \( n = 92 \); pet: pet dogs \( n = 51 \); Se: sensitivity; Sp: specificity.
farm dogs (5.9%). No estimates were possible for the pet dog group, partly due to the small number of animals involved, but possibly also because there were indications that two subgroups were involved, as shown by the posterior distribution for the IFAT specificity (Fig. 1).

IFAT sensitivity was equivalent in the three groups. However, IFAT specificity was considerably lowered in the farm dog group (65.2% versus 94.5% for the stray dogs). Sensitivity and specificity of DAT are equivalent in the three groups, taking into account the very wide credibility intervals. The same can be said for PaGIA, with the exception of the farm dogs where a higher specificity was obtained (87.5% versus 63.3% and 67.3% for stray dogs and national guard dogs respectively). DAT and PaGIA are less sensitive than IFAT, irrespective of the group.

A final model encapsulating the three individual group models was developed and run to estimate the credibility intervals for differences between the true prevalence in the respective groups as well as for differences in test characteristics (6). The results are shown in Table 4. A credibility interval with both limits having the same sign (zero not included in the interval) can be interpreted as the equivalent of a significant result in a frequentist approach.

### Table 3
Estimates of prevalence and sensitivity and specificity of the three diagnostic tests used.

<table>
<thead>
<tr>
<th>Group</th>
<th>$p_D$</th>
<th>DIC</th>
<th>Bayes-p</th>
<th>Prev</th>
<th>Se_IFAT</th>
<th>Sp_IFAT</th>
<th>Se_DAT</th>
<th>Sp_DAT</th>
<th>Se_PaGIA</th>
<th>Sp_PaGIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stray</td>
<td>4.078</td>
<td>37.471</td>
<td>0.587</td>
<td>0.117</td>
<td>0.947</td>
<td>0.945</td>
<td>0.658</td>
<td>0.940</td>
<td>0.787</td>
<td>0.633</td>
</tr>
<tr>
<td>Farm</td>
<td>3.506</td>
<td>27.806</td>
<td>0.434</td>
<td>0.059</td>
<td>0.949</td>
<td>0.652</td>
<td>0.707</td>
<td>0.951</td>
<td>0.660</td>
<td>0.875</td>
</tr>
<tr>
<td>Guard</td>
<td>4.063</td>
<td>32.633</td>
<td>0.406</td>
<td>0.097</td>
<td>0.897</td>
<td>0.888</td>
<td>0.570</td>
<td>0.848</td>
<td>0.648</td>
<td>0.673</td>
</tr>
</tbody>
</table>

### Table 4
Differences in prevalence and in tests sensitivities and specificities between the three groups of dogs: stray, farm and police dogs. Italic indicate differences where the 95% credibility interval does not include 0 and are thus equivalent to a significant difference at 0.05.

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<td>0.057</td>
<td>-0.038</td>
<td>-0.002</td>
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<td>0.127</td>
<td>0.050</td>
<td>0.138</td>
<td>0.292</td>
<td>-0.111</td>
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<td>0.190</td>
<td>0.079</td>
<td>0.369</td>
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<td>0.057</td>
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<td>-0.079</td>
<td>-0.369</td>
<td>0.436</td>
<td>0.167</td>
<td>0.462</td>
<td>0.373</td>
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<td>0.144</td>
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prev: prevalence; Se: Sensitivity; Sp: Specificity; stray: stray dogs, farm: farm dogs; guard: national guard dogs; IFAT: immunofluorescence antibody test; DAT: direct agglutination test; PaGIA: particle gel immunoassay test; lower 95%: lower limit of 95% credibility interval; upper 95%: upper limit of 95% credibility interval.

*Problem with Sp_IFAT, which shows there are two subpopulations.

**Fig. 1.** Posterior distribution for IFAT specificity in pet dog group.

**4. Discussion**

The absence of a gold standard (perfect test with sensitivity and specificity equal to one) and uncertainty about the actual values of sensitivity and specificity of any one test in any one particular situation makes it imperative to use several tests when trying to estimate the prevalence of infection (or disease) in a particular population in a particular area (Berkvens et al., 2006).
Boelaert et al. (1999a) concluded that IFAT could be considered a gold standard for the detection of CL caused by *L. infantum* in a group of 151 stray dogs in Tunisia. Considering IFAT to be a gold standard for the current population of dogs led to various unexpected observations, the most obvious of which was the low sensitivity of DAT in all groups (11.4–44.1%). This raised doubts as to whether IFAT could really be considered a gold standard in the current situation and led to prior distributions for sensitivity and specificity in the domain [0.9–1], thereby allowing the possibility that IFAT is not a gold standard in the given circumstances.

Stray dogs (73% mixed-breed), showed the highest estimated true prevalence (11.7%). This could be related to their outdoor living habits, which increases time of exposure to infected sand flies, and they may be an easier target for infection and sandfly biting due to their precarious physical conditions (Amusategui et al., 2004; Cortes et al., 2007).

The farm dogs are mainly hunting dogs (73%). The true prevalence estimated in this group is the lowest (estimated true prevalence = 5.9%). This is in agreement with (Martín-Sánchez et al., 2009), where the seroprevalence of the *Canine leishmaniasis* in the hunting dogs was lower (15%) than in guard dogs and sheepdogs (31.3%). Paradies et al. (2006) report an annual incidence rate of 6.5% in farm dogs versus 13.1% in kennel dogs.

The intermediate prevalence found for national guard dogs (estimated true prevalence = 9.7%) may be the product of better conditions of life, the habitat (dogs kept together in kennels) and the breed of the animals (predominantly German Shepherds, a breed known to be particularly susceptible to the disease (Abranches et al., 1991; Miranda et al., 2008)). Sharing residence with one or more seropositive dogs could increase the risk for the other dogs to become seropositive (Alonso et al., 2009), especially as the centre of the dog-handling team is located in a wooded area of Algiers. Rossi et al. (2007) showed that the cumulative density (number of specimens/m² of sticky trap/two nights) of *P. perniciosus*, vector of *L. infantum*, was significantly higher in green vegetated environments (forest) compared to strict urban environments.

The difference in age between the three groups may also explain differences in prevalence. *L. infantum* infection is much less frequent in young animals than in adults (Rami et al., 2003; Amusategui et al., 2004; Miró et al., 2007; Martín-Sánchez et al., 2009). This could be related to the increase of time of exposure of dogs to phlebotomines (Zivičnjak et al., 2005): the older an animal, the longer it will have been exposed to sandflies and the higher the probability of having been bitten by an infected female sandfly (Martín-Sánchez et al., 2009). Seventy-eight percent of the farm dogs were less than 4 years old, whereas 66% of the national guard dogs were more than 4 years. The exact age of the stray dogs was more difficult to determine, but the majority (77%) were adults.

The analysis in the three groups showed that IFAT is the most sensitive test, though not a gold standard (sensitivity always lower than one). This lower sensitivity could be related to the fact that most of the dogs investigated were apparently in good health. Despite this observation, IFAT remains the test of choice with a very good capacity to detect low levels of antibodies after relatively recent infections (Boelaert et al., 2004). The rK39 antigen is an indicator of active disease. Sera from early or self-healing infected subjects are generally nonreactive with rK39 (Badaró et al., 1996; Mettler et al., 2005). This is corroborated by the sensitivity of PaGIA, which is of 78.7% in the group of the stray dogs and lower in farm and national guard dogs (66% and 64.8%, respectively).

However, the results are not so unequivocal when considering the relative test specificities in the different groups. IFAT proved less specific in the farm dog group. This might be explained by cross-reactions with (e.g.) *Sarcocystis cruzi (bovi-canis)* (http://web.ics.purdue.edu/jramosva/, click on IHC Tests and then on Leishmania), the causative agent of bovine sarcocystosis for which the dog is the final host (http://www.cfsph.iastate.edu/Factsheets/pdfs/sarcocystosis.pdf). Investigations into the presence of sarcocystosis of domestic animals in Algeria showed 67.4% positive results in cattle, within which 61% of the muscle probes were positive for *S. cruzi* (Nedjari, 2003). In the case of PaGIA, a considerable drop in specificity was noted in the stray dogs group. Mettler et al. (2005) reported cross-reactions with *Hepatozoon canis* and *Neospora caninum*, which they observed in Leishmania-free dogs.

The posterior density distribution for the IFAT specificity in the pet dog group hints at the existence of two subpopulations. However, the small sample size (*n* = 65) and the fact that there were no observations for some of the test result combinations, thereby preventing estimation of certain parameters, precludes the re-categorisation of these animals (e.g.) in function of age or environment where the animals are kept. These factors may influence the duration of exposure of the animals (Abranches et al., 1991; Amelia et al., 1995). It should also be noted that fourteen of 65 pet dogs were sampled in a dog school, a factor that may also increase the chance of infection. Future ethological studies are needed to explain this finding.

The results of the current study demonstrate the variability of test characteristics (sensitivity and specificity) in different situations (in this case different dog groups) and underline the danger of using standard values, either provided by the test kit manufacturer or published by other researchers, without verifying their appropriateness for the specific purposes (e.g. appropriateness for population in question). The present study found differences especially on the side of test specificity of IFAT and PaGIA that might be explained by cross-reactions with other disease causing agents. The present results do not permit an unambiguous choice of explanation for the observed differences, but some hypotheses are offered. More specific field research is required to elucidate these mechanisms.

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Appendix A. Final Bayesian model run in Winbugs

```c
model
{
result[1:8] ~ dmulti(pr[1:8], n)
result1[1:8] ~ dmulti(pr1[1:8], n2)
result3[1:8] ~ dmulti(pr2[1:8], n3)
}
```
\begin{verbatim}
        (1-th2[6])*th2[13]
        (1-th2[6])*th2[13]
        th2[6]*(1-th2[12])
        th2[6]*th2[12]

th[1] ~ dunif(0, 0.5)
th[2] ~ dunif(0.9, 1)
th[3] ~ dunif(0.9, 1)

th[4] ~ dunif(0.5, 1)

th[5] ~ dunif(.95, 1)

th[6] ~ dunif(.4, 1)

th[7] ~ dunif(.2, 1)

th[8] ~ dunif(.2, 1)

th[9] ~ dunif(.3, 1)

th[10] ~ dunif(.2, 1)


th[12] ~ dunif(.5, 1)

th[13] ~ dunif(.2, 1)

th[14] ~ dunif(.2, 1)

th[15] ~ dunif(0, 1)

th[1] ~ dunif(0, 1)

th[2] ~ dunif(0.9, 1)

th[3] ~ dunif(0.6, 1)

th[4] ~ dunif(0.5, 1)

th[5] ~ dunif(0, 1)

th[6] ~ dunif(0.8, 1)

th[7] ~ dunif(0, 1)

th[8] ~ dunif(0.5, 1)

th[9] ~ dunif(0, 1)

th[10] ~ dunif(0, 1)

th[11] ~ dunif(0, 1)

th[12] ~ dunif(0.8, 1)

th[13] ~ dunif(0, 1)

th[14] ~ dunif(0, 1)

th[15] ~ dunif(0, 1)

th[2] ~ dunif(0, 0.5)

th[2] ~ dunif(0.8, 1)

th[2] ~ dunif(0.6, 1)

th[2] ~ dunif(0.3, 1)

th[2] ~ dunif(0.9, 1)

th[2] ~ dunif(0.8, 1)

th[2] ~ dunif(0, 1)

th[2] ~ dunif(0.765, 1)

th[2] ~ dunif(0, 1)
\end{verbatim}
th2[10] ~ dunif(0,1)
th2[11] ~ dunif(0,1)
th2[12] ~ dunif(.5,1)
th2[13] ~ dunif(0,1)
th2[14] ~ dunif(0,1)
th2[15] ~ dunif(0,1)


for (i in 1:8)
{
  d[i] <- result[i]*log(max(result[i],1)/(pr[i]*n))
}

G0 <- 2*sum(d[])
result2[1:8] ~ dmulti(pr[1:8],n)
for (i in 1:8)
{
  d2[i] <- result2[i]*log(max(result2[i],1)/(pr[i]*n))
}

Gt <- 2*sum(d2[])
bayesp <- step(G0 - Gt)

for (i in 1:8)
{
  d1[i] <- result1[i]*log(max(result1[i],1)/(pr1[i]*n2))
}


Alonso, F., Giménez Font, P., Manchón, M., Ruiz de Ybáñez, R., Segovia, M., Berriatua, E., 2009. Geographical variation and factors associated to


