Prevalence of antibody seroconversion to Toxoplasma gondii in uveitis and non-uveitis dogs

Goran Kosec, Biljana Hacin, Philip G Sansom, Gereth Weaver, Emma Dewhurst, James W Carter

ABSTRACT

Objectives To evaluate the prevalence of seroconversion to Toxoplasma gondii in dogs with uveitis and dogs without uveitis.

Methods In total, 135 dogs were evaluated: 51 dogs were diagnosed with uveitis, and 84 dogs were without uveitis. Latex agglutination tests were performed on all sera, and the results were evaluated.

Results Overall, 7.8 and 6.0 per cent of sera were positive for the presence of anti-T gondii antibodies (dilution ≥1:64) in the groups with uveitis and non-uveitis dogs, respectively. The frequency distribution of variables (positive and negative results in the uveitis and the non-uveitis group of dogs) was tested with Fisher's exact test. There was no statistically significant difference between groups (P=0.73).

Clinical significance These findings suggest that evidence of exposure to T gondii was not significantly different between uveitis and non-uveitis groups of dogs and that the possible association between exposure to T gondii and canine uveitis requires further investigation. This study is the first to report the seroprevalence of anti-T gondii antibodies in dogs in the UK population and the first to report the seroprevalence of anti-T gondii antibodies in dogs with uveitis.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that is remarkable in its ability to infect any nucleated cell in all warm-blooded animals. Animals may become infected by one of three means: ingestion of sporulated oocysts (sporozoites) from an environment contaminated by cat faeces; ingestion of tissue cysts or bradyzoites from carnivores of animals that have been infected with T gondii; or congenital transmission of the organism. The pathogenesis of toxoplasmosis has historically been considered to be the result of a direct cytopathic effect, that is, cellular necrosis by intracellular growth of the organism T gondii, as the organism produces no toxins that damage cells. The definitive hosts for T gondii are domestic cats and other Felidae where oocysts may be shed in faeces. Intermediate hosts are probably all warm-blooded animals including most livestock, and humans. In these intermediate hosts, the organism replicates in the tissues, but a gut life cycle does not occur, and no faecal oocysts are shed.

A study by Lappin et al showed that anterior uveitis developed in serologically negative cats 84 days after oral inoculation with T gondii. In the same study, four cats presented with retinal scars at 15 days after inoculation, with similar observations at 80 days after inoculation. In dogs, keratoconjunctivitis, episcleritis, scleritis, optic neuritis, chorioiditis, retinitis, ciliary epithelial hyperplasia, anterior uveitis, iridocyclitis, posterior uveitis and panuveitis have all been reported in association with toxoplasmosis.

In humans, it is believed that the majority of horizontal transmissions are caused by ingestion of one of the two persistent stages of T gondii, that is, tissue cysts in infected meat or offal (viscera) and oocysts in food or water contaminated with feline faeces. Sero-prevalence in humans is affected by many factors. Climatic factors play a significant role in oocyst survival in the environment and hence alter infection rates in meat-producing animals. Lifestyle factors explain a large part of the variation in human seroprevalence, including dietary habits (methods of cooking meat, hand washing, kinds of meat and vegetables consumed, and so on), and economic, social or cultural habits including water quality and sanitation coverage. In humans with ocular toxoplasmosis, an active lesion presents as grey-white foci of retinal necrosis with adjacent choroiditis, vasculitis, haemorrhage and vitritis. Cicatrisation occurs from the periphery towards the centre, with variable pigmented changes. Anterior uveitis is another common finding, with mutton-fat keratic precipitates, cells and aqueous flare, and posterior synechiae.
The seroprevalence of anti-*T. gondii* antibodies in dogs varies considerably between studies, ranging from 1.8 to 96.3 per cent. Currently there are no reports of the seroprevalence of anti-*T. gondii* antibodies in dogs in the UK.

The paucity of clinical reports of ocular toxoplasmosis in dogs suggests that it is not a common cause of clinically important ocular inflammatory disease. *Toxoplasma* serology is often a routine part of clinical investigation for canine uveitis, but in the authors’ experience *T. gondii* has rarely been proved to be the cause of clinical disease. This study was designed to investigate *T. gondii* seroprevalence and its association with canine uveitis.

**MATERIALS AND METHODS**

**Animals and inclusion criteria**

The study was approved by the Recognised Veterinary Practice (RVP) Subcommittee of The Royal College of Veterinary Surgeons and performed at South Devon Referrals, Abbotskerswell, UK. In accordance with the RVP guidance, only waste blood samples were included for the purpose of the study.

Latex agglutination tests (LAT), Toxoreagent RST701 (Eiken Chemical, Tokyo, Japan [Mast Diagnostics, Bootle, UK]) results in dogs that presented to the referral centre, were collected both retrospectively (June 2005 to December 2014) and prospectively (January 2015 to December 2015).

All LATs were run by the same referral veterinary laboratory, Axiom Veterinary Laboratories (the laboratory), for the presence of anti-*T. gondii* antibodies. Dogs were divided into two groups: those with known uveitis (uveitis group) and those that presented to the referral centre for other disease processes (non-uveitis group).

To meet the inclusion criteria for the uveitis group, dogs had to present with at least three of the following clinical signs for acute-onset uveitis: corneal oedema, keratic precipitates, ocular hyperaemia (conjunctival hyperaemia, scleral blood vessel congestion), aqueous flare, hypopyon, hyphema, miosis, vitreous cellularity, chorioretinitis and hypotony (defined as intraocular flare, hypopyon, hyphema, miosis, vitreous cellularity, hyperaemia, scleral blood vessel congestion), aqueous keratic precipitates, ocular hyperaemia (conjunctival dogs had to present with at least three of the following for other disease processes (non-uveitis group).

Both groups (uveitis and non-uveitis groups) consisted of dogs from the retrospective (June 2005 to December 2014) and prospective periods (January 2015 to December 2015).

Dogs included in the retrospective part of the study had undergone an ophthalmic examination performed by the same boarded ophthalmologist (JWC). All dogs in the prospective part of the study were examined by the same veterinary ophthalmology resident (GK) and supervised by the same boarded ophthalmologist (JWC) as in the retrospective part of the study.

**Serum samples**

Throughout the entire study period, blood samples were collected by jugular venipuncture and handled in accordance with the good laboratory practice guidance and practice standard operating procedure. The samples were left to clot at room temperature between six and seven hours. Serum was then separated from the clot by centrifugation at 1800 g for 10 minutes and if not immediately dispatched to the laboratory, stored at −20°C until dispatched.

**Serological assay**

Serological detection of total immunoglobulin and IgG to *T. gondii* was performed at an external veterinary laboratory using a commercial LAT and carried out according to the manufacturer’s instructions. The test was performed in microtitre plates with V-shaped wells. Each serum sample was diluted 1:16 with the diluent buffer, and 25 microlitres of the diluted test sample was further diluted with an equal volume of buffer solution, generating serial twofold dilutions from 1:16 to 1:1024. Latex reagent (0.1 per cent *Toxoplasma* antigen-sensitised polystyrene latex suspension) was added to the sample wells and compared with the positive control (anti-toxoplasma-containing swine serum). The plates were analysed in daylight against a dark background. A positive result was determined when agglutination was detected and compared with a positive control. The cut-off titre was taken as 1:64 according to the manufacturer’s instructions, and an endpoint titre was established for positive samples. A negative reaction was indicated by sunken latex on the bottom of the well. To detect IgG only, 2-mercaptoethanol was added to an aliquot of test serum to remove all antibodies of the IgM class.

The results for total immunoglobulin titre and an IgG class titre were assessed and compared for both the uveitis and non-uveitis groups. Although the presence of IgM may be inferred if total immunoglobulin titre is greater than the IgG titre, the addition of 2-mercaptoethanol removes all antibodies of the IgM class and not just those specific to *Toxoplasma*. This means that it is not possible to calculate a specific *T. gondii* IgM titre. Fisher’s exact test was used to test for differences in the seroprevalence rates of dogs in the uveitis and non-uveitis groups. For statistical analyses, *P*<0.05 was considered to indicate significance.

**RESULTS**

**Study population**

In total, 135 dogs met the inclusion criteria for the study. The dogs were assigned to an uveitis group consisting of 51 dogs or a non-uveitis group consisting of 84 dogs.
The male/female ratio was 1:2.2 in the uveitis group compared with 1:0.9 in the non-uveitis group. Age presentation between male and female dogs was similar in the uveitis group; however, the average age of male dogs was greater than that of females in the non-uveitis group. Testing for differences in seroprevalence between male and female dogs with and without uveitis, including age as a covariate, was not possible with the small sample population, and had not been an aim of the study. Comparison of age and sex distribution for the uveitis and non-uveitis groups is summarised in table 1.

The most common breeds in the uveitis group were labrador retriever (18 per cent) and golden retriever (12 per cent), followed by border collie, springer spaniel and crossbreed (8 per cent). The most common breeds in the non-uveitis group were Jack Russell terrier (17 per cent), labrador retriever (10 per cent) and border collie (8 per cent).

Serology

The overall seroprevalence of anti-*T gondii* antibodies in this study was found to be 6.7 per cent (n=9).

The results of serological testing as per LAT revealed dogs positive for anti-*T gondii* antibodies (≥1:64 total immunoglobulin). The seroprevalence results of the LAT for the uveitis and non-uveitis groups are summarised in table 2.

Overall, there were nine dogs with a total immunoglobulin titre of greater than or equal to the 1:64 cut-off: four in the uveitis group and five in the non-uveitis group. Of the nine dogs, with a total immunoglobulin titre of greater than or equal to the 1:64 cut-off, six were positive for an IgG titre greater or equal to our cut-off of 1:64 (one in the uveitis group and four in the non-uveitis group).

Of the same group of nine dogs, there were four dogs present with identical total immunoglobulin and IgG titres (one in the uveitis group and three in the non-uveitis group). Therefore, by inference there was no detectable level of other immunoglobulins direct to *T gondii* in these dogs with IgM being the major component. This does not preclude these patients from having active clinical infection with *T gondii* but may suggest that they are not acute cases or may have retained a positive serological titre following previous exposure. On the other hand, there were five dogs present (three in the uveitis group and one in the non-uveitis group) with higher total immunoglobulin titres in comparison to IgG.

In the uveitis group, 7.8 per cent (n=4) of dogs tested positive for anti-*T gondii* antibodies. Comparison of clinical signs in the uveitis group is summarised in table 3.

In the non-uveitis group, 6.0 per cent (n=5) of dogs tested positive for anti-*T gondii* antibodies. Comparison of clinical signs in the non-uveitis group is summarised in table 4.

The most common clinical signs of uveitis are summarised in table 5. These included ocular hyperaemia (65.0 per cent; n=33), aqueous flare (61.0 per cent; n=31) and corneal oedema (37.0 per cent; n=19).
was present in the left eye (27.5 per cent; n=14) of dogs, in the right eye (21.5 per cent; n=11) and bilaterally (51.0 per cent; n=26).

Statistics

Table 6 shows the comparison of the seroprevalence rates of the uveitis and non-uveitis groups. Fisher’s exact test of the difference in seroprevalence between the uveitis and non-uveitis groups of dogs yielded a P value of 0.729. This result is not significant at the 5 per cent level, indicating that there was no significant difference between the groups with or without uveitis.

Age distribution is not statistically significant in this study population (Fisher’s exact test P=0.33).

DISCUSSION

The main objective of this study was to demonstrate whether LAT serology for the detection of anti-\( T \) \( gondii \) antibodies was beneficial in dogs suspected of ocular toxoplasmosis. In the population studied there was no significant difference at the 5 per cent level in the seroprevalence of \( T \) \( gondii \) for uveitis and non-uveitis dogs as per LAT serology. There are two key points one should take into consideration when interpreting anti-\( T \) \( gondii \) antibody test results in dogs. First, no single serological test exists that can definitively confirm toxoplasmosis. Secondly, comparison of serology results between the different tests is difficult due to the variation in serological techniques and the choice of threshold for positive cut-off values. However, combining tests significantly improves the diagnostic sensitivity. In this study, the LAT was selected as the method of choice due to the availability and established protocol for dogs by the laboratory. Furthermore, the laboratory regularly assesses the appropriateness of this kit for determining antibody titres in dogs through participation in an external quality assurance scheme run by the Veterinary Laboratory Association through their Quality Assurance Program in the USA.

Different tests are available when testing for the presence of \( T \) \( gondii \). Indirect haemagglutination tests, fluorescent antibody tests, LATs, modified agglutination tests, ELISA, Sabin-Feldman dye tests and others have been described in different publications. The dye test, first developed by Sabin and Feldman in 1948, has been considered the gold standard for the detection of anti-\( T \) \( gondii \) antibodies in humans. The test is technically difficult to perform and hazardous because live virulent tachyzoites are used. The LAT showed adequate performance in sensitivity and specificity for the detection of anti-\( T \) \( gondii \) antibodies in humans but low performance in sheep. It has been evaluated for diagnosis of toxoplasma infection in pigs, cats, dogs and rodents, and a positive titre is defined as greater than or equal to 1:64 dilution. Application of the LAT for the diagnosis of toxoplasma infection in dogs was not proved successful in 1981. The agglutination test is based on the agglutination of organisms in the presence of a specific immunoglobulin in the patients’ sera and does not require species-specific conjugates. Agglutination tests can be therefore performed on sera from any species without modification.

The LAT test reports total immunoglobulin as it does not differentiate between the different immunoglobulin classes. In theory, the majority of the immunoglobulin in serum consists of IgG and IgM. By adding 2-mercaptopetoethanol at a defined concentration to the sample, it is possible to denature all antibodies of the IgM class and report IgG only. IgG is more resistant to degradation by 2-mercaptoethanol than other immunoglobulin classes. The LAT results (table 2) show the highest positive titre for total immunoglobulin and for IgG after the sample was treated with 2-mercaptoethanol. The study reports

### Table 2
<table>
<thead>
<tr>
<th>Positive total immunoglobulin at dilution</th>
<th>1:64</th>
<th>1:256</th>
<th>1:128</th>
<th>1:256</th>
<th>1:1024</th>
<th>1:1024</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive IgG at dilution</td>
<td>1:32</td>
<td>1:64</td>
<td>1:128</td>
<td>1:256</td>
<td>1:512</td>
<td>1:1024</td>
</tr>
<tr>
<td>Number of dogs positive for the LAT in uveitis group</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Number of dogs positive for the LAT in non-uveitis group</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

LAT, latex agglutination test.

### Table 3
<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral uveitis</td>
<td>1</td>
</tr>
<tr>
<td>Bilateral hyphema bilateral hyphaemia and anterior uveitis due to immune-mediated thrombocytopenia</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral episcleritis and anterior uveitis</td>
<td>1</td>
</tr>
</tbody>
</table>

n, number of animals.

### Table 4
<table>
<thead>
<tr>
<th>Clinical signs</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral episcleritis</td>
<td>1</td>
</tr>
<tr>
<td>Optic neuropathy</td>
<td>1</td>
</tr>
<tr>
<td>Lameness</td>
<td>1</td>
</tr>
<tr>
<td>Unilateral cataract formation</td>
<td>1</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1</td>
</tr>
</tbody>
</table>

n, number of animals.
nine (6.7 per cent) positive cases, this comprised four (7.8 per cent) in the uveitis and five (6 per cent) in the non-uveitis group. Fisher’s exact test gives a P value of 0.73. This supports the view of there being no significant difference in the seroprevalence to anti-\( T\) gondii antibody between the two groups at the 5 per cent level.

Demonstrating rising IgG titres in serum samples obtained at three-week intervals in the absence of any specific therapy is suggestive in humans of an infection acquired less than two months before the first sample, but one must be aware that treatment may also reduce or abolish the increase in IgG titres.\(^8\) The study design does not report paired samples, therefore the results are reporting seroprevalence to specific antibodies in uveitis in random non-uveitis dogs only, rather than rising titres, which could assign \( T\) gondii as a causative agent for toxoplasmosis.

IgM antibodies are produced in humans during the first week following infection and reach a plateau within one month. IgM antibodies usually decrease after one to six months; they become negative in 25 per cent of human patients within less than seven months but more commonly remain detectable for a year or longer.\(^8\) Regardless of the methodology used for detection, IgG synthesis reaches a plateau within two or three months, decreases more or less rapidly and persists lifelong at residual titres, which are highly variable among patients.\(^8\)

To the best of the authors’ knowledge there are no published studies of anti-\( T\) gondii antibody dynamics in the dog and currently there is no single serological test that can definitively confirm toxoplasmosis.

Due to problems with IgM not being a reliable marker of a recent infection in humans, IgG avidity is used. IgG avidity demonstrates that antibody binding becomes stronger the longer the host has been infected.\(^8\) However, this phenomenon has not yet been demonstrated in dogs.

The diagnostic LAT test used within this study will determine the total immunoglobulin and IgG levels within a sample, but does not give a specific titre value for IgM. This is because the 2-mercaptoethanol used within this test has the ability to remove all antibodies of the IgM class from the serum and not just that specific for \( T\) gondii, hence preventing the summation of the projected IgM value with the IgG to give the total immunoglobulin. A study by Cabral et al using a LAT test added a second stage using 2-mercaptoethanol in samples which had a positive titre of equal to or greater than 1:512.\(^27\) This study gave an indication of IgM presence in these samples but only as an inference of the change in titre after the addition of 2-mercaptoethanol. No actual values were given.

One method to demonstrate active local infection to \( T\) gondii is by comparing local antibody production with serum antibody levels. This has been confirmed in cats experimentally infected with \( T\) gondii by demonstrating the Witmer-Goldmann coefficient, or the so-called ‘\( C\) value’.\(^28\)\(^29\) The \( C\) value is the ratio of aqueous antibody titre to serum titre.\(^28\)\(^29\) A \( C\) value greater than 1 indicates that an intraocular infectious agent is causing iridal plasma cells to produce antibodies, thus demonstrating that an infectious agent is causing the uveitis rather than merely being a bystander.\(^6\) To date, there have been no publications looking at the Witmer-Goldmann coefficient using \( T\) gondii titres within the dog, and it has only been used in a single publication in a group of dogs in Brazil that were naturally infected with \( Leishmania chagasi\).\(^30\)

The authors have been unable to locate any published study showing the seroprevalence of anti-\( T\) gondii antibodies in dogs with uveitis. In a study of cats with uveitis, 18.3 per cent of them were found to be seropositive for anti-\( T\) gondii antibodies.\(^31\) A study of 102 dogs with uveitis performed at North Carolina State University Veterinary Teaching Hospital does not report the seroprevalence rates of anti-\( T\) gondii antibodies despite 57.8 per cent of dogs being diagnosed with idiopathic/immune-mediated uveitis.\(^32\)\(^35\)

A comparison of serology results between studies using the LAT for detection of anti-\( T\) gondii antibodies shows higher (6.7 per cent) seroprevalence of anti-\( T\) gondii antibodies in the study than in one performed in Tokyo, 1.8–1.9 per cent\(^11\); but lower than in Thailand, 11 per cent\(^33\); Korea, 12.8 per cent\(^34\); Taiwan (China), 19.6 per cent\(^36\) and 20.1 per cent\(^36\); and Nigeria, 25 per cent\(^37\).

<table>
<thead>
<tr>
<th>Table 5</th>
<th>The most common clinical signs of uveitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical signs of uveitis</td>
<td>n</td>
</tr>
<tr>
<td>Ocular hyperaemia</td>
<td>33</td>
</tr>
<tr>
<td>Aqueous flare</td>
<td>31</td>
</tr>
<tr>
<td>Corneal oedema</td>
<td>19</td>
</tr>
<tr>
<td>Ocular hypotony (IOP&lt;5)</td>
<td>16</td>
</tr>
<tr>
<td>Fibrin, hyphaema or hypopyon</td>
<td>13</td>
</tr>
<tr>
<td>Miosis</td>
<td>9</td>
</tr>
<tr>
<td>Retinal detachment, chorioretinitis</td>
<td>11</td>
</tr>
<tr>
<td>Keratic precipitates</td>
<td>7</td>
</tr>
<tr>
<td>Optic neuritis with uveitis</td>
<td>8</td>
</tr>
<tr>
<td>Vitreous cellularity</td>
<td>4</td>
</tr>
</tbody>
</table>

IOP, intraocular pressure.

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Comparison of the seroprevalence rates of anti-Toxoplasma gondii antibodies between the uveitis and non-uveitis groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>Positive</td>
</tr>
<tr>
<td>Sum of both uveitis and non-uveitis groups</td>
<td></td>
</tr>
<tr>
<td>Uveitis group</td>
<td>4</td>
</tr>
<tr>
<td>Non-uveitis group</td>
<td>5</td>
</tr>
<tr>
<td>Marginal column totals</td>
<td>9</td>
</tr>
</tbody>
</table>

Fisher’s exact test gives a P value of 0.73, which is not significant at the 5% level.
One reason for the low seroprevalence of anti-*T. gondii* antibodies in this study could be associated with the living conditions and diet of the dogs. Worming status and lifestyle of the dogs were not collected; however, all dogs were family owned with a good welfare background. Further investigation would benefit from a questionnaire involving feeding habits (commercial diet v Biologically Appropriate Raw Food diet or home prepared food) and worming history including agent used. In humans, eating raw or undercooked meat increases the risk of infection but was not associated with development of ocular toxoplasmosis.\(^\text{38}\) The infection rate was higher in strays, guard dogs, or dogs fed on a raw meat diet than in household dogs.\(^\text{34 - 36}\) There was no information gathered about the lifestyle of the dog (working or pet dogs) in this study.

There were not sufficient data to test for differences in seroprevalence or susceptibility to uveitis across breeds. However, the fact that labrador retrievers, golden retrievers and border collies were among the most common breeds in both groups suggests that the results should not be strongly biased by unknown genetic factors.

Low dose of infective agent at the time of the infection, age of the host at the time of infection and an infection with a different infective stage of the organism (tachyzoite, oocyst, cyst) may also cause false negative results.\(^\text{43}\) Furthermore, organism strain may have an important role in seroconversion; for example, kittens from queens infected with ME-49 and Maggie strains of *T. gondii* developed chorioretinitis consistent with ocular toxoplasmosis but failed to produce detectable anti-*T. gondii* antibodies.\(^\text{45}\) The ability of *T. gondii* to cause ocular disease without systemic disease is poorly understood.\(^\text{41}\) Immune-mediated uveitis without systemic disease may be due to exposure to *T. gondii* antigens at non-ocular locations and homing of activated immune cells to ocular tissues, molecular mimicry, circulating antigens or immune complexes from non-ocular sites of parasite replication and a non-specific increase in immune response.\(^\text{2, 4}\)

The time frame of the retrospective part of this study was 10.5 years; however, the time frame of the prospective part was only one year. There may be a difference in seroprevalence rates to anti-*T. gondii* antibodies in the past decade that could influence the current results. However, a study of shelter dogs in Tokyo showed only a mild increase in seroprevalence rates of anti-*T. gondii* antibodies over the past decade.\(^\text{11}\) To our knowledge, there are no reports of seroprevalence rates for anti-*T. gondii* antibodies in dogs in the UK. Our overall seroprevalence rate for anti-*T. gondii* antibodies may reflect a seroprevalence in the south-west region of the UK and may differ from the seroprevalence rate in the overall dog population in the UK. Furthermore, the relatively small sample size in this study limits the power of the statistical tests used.

Only the prospective part of the study was closely monitored and standardised; however, for all blood collections and handling in the retrospective part of the study, the good laboratory practice guidance was followed at all times to ensure the generation of high-quality and reliable test results.

Despite our results showing no correlation between seroprevalence of anti-*T. gondii* antibodies in dogs with and without uveitis, *T. gondii* still remains one of the potential causes for uveitis. The clinical importance of ocular toxoplasmosis for canine uveitis needs to be further evaluated. Due to the variety of test kits for *T. gondii* antibody detection, information for cut-off values for each type of test is crucial to properly assign the result.

Despite showing that there was no significant difference between the groups, the statistical power was fairly low and indicated that given the low incidence rates, a much larger sample population would be ideal.

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**Data sharing statement** No additional data are available.

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