



Review

Research into *Neospora caninum*—What Have We Learnt in the Last Thirty Years?

Michael P. Reichel ^{1,2,*} , Lloyd C. Wahl ¹ and John T. Ellis ³ 

¹ Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong, China; lloyd.wahl@cityu.edu.hk

² Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, USA

³ School of Life Sciences, University of Technology Sydney, P.O. Box 123, Broadway, NSW 2007, Australia; John.Ellis@uts.edu.au

* Correspondence: mpr97@cornell.edu

Received: 2 May 2020; Accepted: 21 June 2020; Published: 23 June 2020



Abstract: Background: *Neospora caninum* has been recognised world-wide, first as a disease of dogs, then as an important cause of abortions in cattle for the past thirty years. Over that time period, there have been improvements in the diagnosis of infection and abortion, new tests have been developed and validated, and it is timely to review progress to date. Methods: Bibliometric methods were used to identify major trends and research topics present in the published literature on *N. caninum*. The tools used were SWIFT-Review, VOSviewer and SciMAT, along with the published papers found in the MEDLINE, Dimensions and Web of Science databases. A systematic review of the published *Neospora* literature ($n = 2933$) was also carried out via MEDLINE and systematically appraised for publications relevant to the pathogenesis, pathology and diagnosis of *Neospora* abortions. Results: A total of 92 publications were included in the final analysis and grouped into four main time periods. In these four different time periods, the main research themes were “dogs”, “abortion”, “seroprevalence” and “infection”. Diagnostics, including PCR, dominated the first two time periods, with an increased focus on transmission and abortions, and its risk factors in cattle. Conclusions: Longitudinal analyses indicated that the main themes were consistently investigated over the last 30 years through a wide range of studies, with evolving emphasis initially on dogs and diagnostic test development, followed by application to cattle, the identification of the risk factors leading to abortion, and in the latter time periods, an understanding of the immunity and a search for vaccines.

Keywords: *Neospora caninum*; dogs; cattle; abortions; diagnosis; pathogenesis; review

1. Introduction

Neospora caninum is a protozoan parasite that was first recognised about 30 years ago as the aetiological cause of abortion in infected cattle [1–3]. *N. caninum* has since become one of the most frequently diagnosed causes of abortions in cattle and is estimated to cause cattle producers world-wide losses exceeding a billion dollars annually [4]. Seroprevalence studies show infection rates vary considerably between and within countries, and between dairy and beef cattle [4]. For example, recovery rates of *N. caninum* from aborted cattle foetuses range from as low as 12% to as high as 42% [5]. There is evidence that *N. caninum* may cause reproductive disruption in small ruminant species, such as sheep and goats [6,7]. There have also been reports of cerebral neosporosis in sheep [8]. The water buffalo (*Bubalus bubalis*) appears to be readily infected with *N. caninum*, however abortions in this bovid are rarely reported [9]. Dogs are the primary definitive host for this parasite in which sexual

replication occurs [10], and they may also be affected clinically [11,12]. Other canids may also act as definitive hosts in parts of the world [13,14].

While we have known *N. caninum* to be a cause of infection and abortions in cattle for thirty years, there has been ongoing discussion about how to best diagnose and prevent a *Neospora* abortion [15], as well as the ways to develop appropriate, practical, economically justifiable and efficacious tools to prevent them. The bulk of publications on the subject is approaching 3000 scientific manuscripts and these present our combined knowledge on the subject. The following presents an analysis of that literature of those three decades in a systematic way, identifies the key steps in our understanding, and documents the progress of the past three decades as well as possible steps for the future.

2. Results

Four different databases were searched for literature using the term “*Neospora*” (Table 1). All databases returned slightly different numbers of papers depending on the search performed. Although a search of Web of Science (WoS) with the term “*Neospora*” returned the highest number of publications, MEDLINE provided a higher number of papers relating to terms of importance to this review (diagnosis, pathology and pathogenesis).

Table 1. Summary of the number of papers identified in searches of different databases.

Search Terms	MEDLINE	Dimensions ¹	Scopus ²	WoS ³	WoS ⁴
<i>Neospora</i>	2379	2905	2998	3068	3470
(“ <i>Neospora</i> ”) AND Diagnosis	1016	329	442	872	882
(“ <i>Neospora</i> ”) AND pathology	430	42	211	80	147
(“ <i>Neospora</i> ”) AND pathogenesis	2010	76	115	143	145

¹ (title and abstract); ² (article, title, keywords); ³ (topic); ⁴ (all fields). Accessed on 25 March 2020.

2.1. Topic Modelling

Topic modelling of the literature from MEDLINE on “*Neospora*” was performed in SWIFT-Review [16]. This approach is helpful in the identification of the major themes present in the literature through the identification of key words included in the topic model. The top 15 topic models (of the 100 returned) are presented in Table 2 and Figure 1. Table 2 includes lay descriptions formulated as an attempt to provide a simple meaning to the topic model as a theme. Most of the topics identified are associated with diagnosis and pathology of infections in cattle and experimental infections in mice. Serology, seroprevalence, molecular diagnostics, disease development and risk analyses are the key themes that dominate.

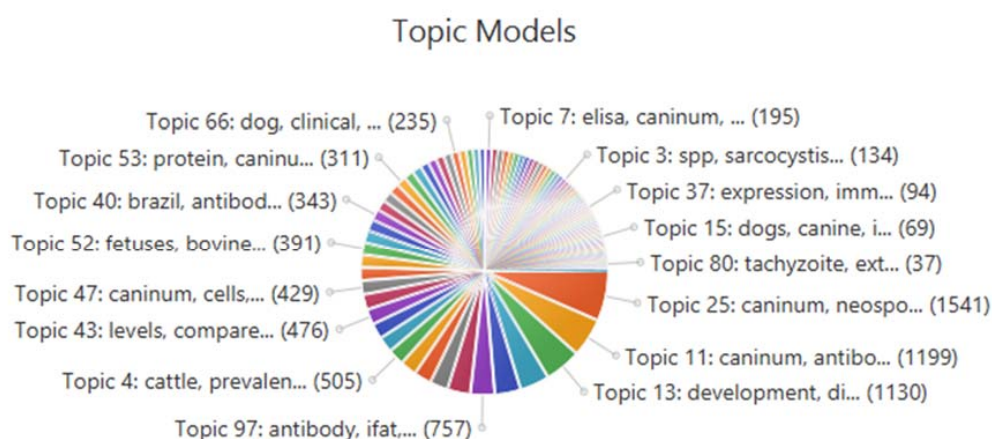


Figure 1. Overview of topic models generated from the MEDLINE dataset using SWIFT-Review.

Table 2. Top 15 topic models identified by SWIFT-Review of “*Neospora*” literature from MEDLINE.

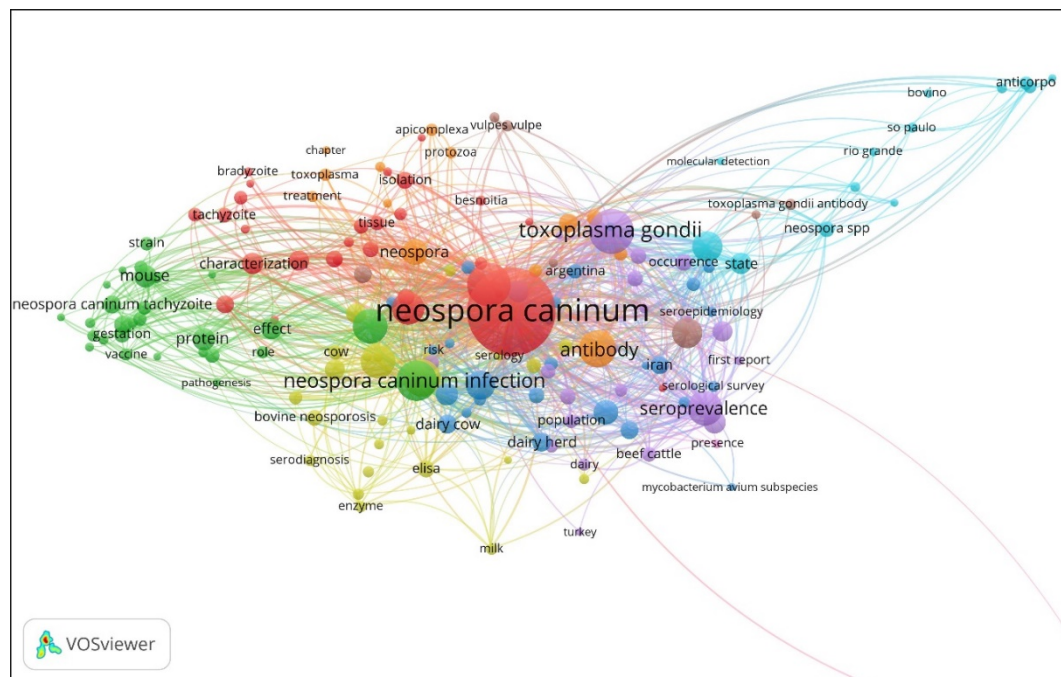
Topic Number	Topic Words	Number of Publications Contributing to Topic Model	Lay Description of Topic
25	<i>caninum</i> , <i>Neospora</i> , cattle, neosporosis, parasite, infection, abortion, bovine, important, disease	1541	<i>Neospora</i> as a cause of abortion in cattle
11	<i>caninum</i> , antibodies, <i>Neospora</i> , seroprevalence, infection, samples, study, positive, prevalence, presence	1199	Seroprevalence of <i>Neospora</i>
13	development, disease, studies, species, including, animal, diseases, vaccine, approach, understanding	1130	Disease development and control
49	<i>gondii</i> , <i>Toxoplasma</i> , <i>caninum</i> , <i>Neospora</i> , parasites, infections, toxoplasmosis, animals, study, species	911	<i>Neospora</i> and <i>Toxoplasma</i> infections in animals
61	<i>caninum</i> , pcr, dna, samples, <i>Neospora</i> , positive, detection, detected, brain, tissues	802	PCR detection of <i>Neospora</i> in animal tissues
97	antibody, ifat, <i>caninum</i> , test, antibodies, indirect, <i>Neospora</i> , serum, fluorescent, samples	757	Immunofluorescent antibody testing
29	<i>caninum</i> , mice, infection, <i>Neospora</i> , tachyzoites, infected, days, inoculated, parasite, experimental	709	Experimental infections in mice
27	<i>caninum</i> , <i>Neospora</i> , lesions, infection, neosporosis, brain, tachyzoites, immunohistochemical, immunohistochemistry, encephalitis	559	Immunohistochemistry and pathology
17	cows, <i>caninum</i> , seropositive, dairy, <i>Neospora</i> , abortion, seronegative, heifers, animals, cattle	540	Serology in cattle
36	risk, factors, farms, seroprevalence, study, seropositivity, analysis, regression, herds, logistic	512	Risk analyses
4	cattle, prevalence, <i>caninum</i> , seroprevalence, herds, dairy, <i>Neospora</i> , infection, beef, herd	505	Seroprevalence of <i>Neospora</i> in cattle
19	test, tests, results, sensitivity, bovine, elisa, serological, sera, specificity, cattle	489	Serological diagnostics for cattle
78	<i>caninum</i> , antigens, antigen, kDa, tachyzoites, <i>Neospora</i> , recognized, tachyzoite, antibodies, monoclonal	483	Identification of antigens
43	levels, compared, study, significant, significantly, observed, results, higher, increased, found	476	Diagnostics for cattle
33	host, parasite, cell, <i>Neospora</i> , parasites, intracellular, <i>caninum</i> , infection, apicomplexan, <i>Toxoplasma</i>	471	<i>Neospora</i> and <i>Toxoplasma</i> infections in animals

2.2. Bibliometric Analysis

A search of the Dimensions database using the term “*Neospora*” returned 2987 publications between 1989 and 2019; references with identical titles were deleted, leaving 2935 publications, which were imported into VOSviewer (www.vosviewer.com, Universiteit Leiden, Leiden, Netherlands, Version 1.6.15) [17] for bibliographic analyses. The main network characteristics obtained from an analysis of co-occurrence of words are shown in Table 3 and Figure 2. Of the 5571 terms present in the paper titles, 167 major terms (nodes) met the minimum occurrence of 10 specified. This data is organised by VOSviewer into nine main clusters, which are summarised in Table 3. These nine clusters represent simply a high-level overview of the main studies that were carried out on *Neospora*. Some are easy to identify in terms of lay description; for example, cluster 2 is primarily about using mouse models to study vaccine development and drug treatment. At least four of the clusters are closely linked to the use of serology in the study of reproductive performance, epidemiology and seroprevalence (clusters 3–5 and 9). Cluster 6 relates to the application of molecular diagnostics to define the disease burden in Brazil; and cluster 8 points to studies on the red fox (*Vulpes vulpes*) as a host.

Table 3. Summary of the word clusters identified using VOSviewer and the Dimensions dataset obtained using a search for the term “*Neospora*”.

Cluster	Cluster/Lay Description	Keywords	Species and Diseases	Countries Mentioned
1	<i>Neospora caninum</i>	Brain, calf, isolation, bradyzoite, detection, isolate, isolation, PCR, oocyst, molecular characterisation	<i>Besnoitia</i> , <i>Hammondia</i> , <i>Hammondia heydorni</i> , <i>Sarcocystis</i>	Japan
2	Mouse models	Experimental infection, gestation, immunisation, placenta, pregnancy, protection, strain, toltrazuril, vaccine, vertical transmission	Balb/C mouse	
3	Reproduction	Abortion, association, cause, evidence, herd, reproductive performance, review, risk, seroepidemiology, serological survey	<i>Bubalus bubalis</i> (water buffalo), dairy cow, <i>Mycobacterium avium</i> subspecies <i>paratuberculosis</i> ,	Argentina, Australia, Iran, New Zealand
4	Seroepidemiology	Anti <i>Neospora caninum</i> antibody, blood, cat, control, diagnosis, elisa, epidemiology, milk, serodiagnosis, transplacental transmission	bovine neosporosis, <i>Toxoplasma gondii</i> infection, toxoplasmosis	
5	Seroprevalence	dairy farm, disease, investigation, <i>Neospora caninum</i> antibody, occurrence, population, risk factor, seroprevalence, serosurvey	Beef cattle, goat, sheep, <i>Toxoplasma gondii</i>	China, Mexico, Brazil, Spain, Turkey
6	Molecular detection in Brazil	Bovino, molecular detection, state	<i>Neospora</i> spp.	Brazilian locations
7	Treatment of cyst-forming coccidia	Antibody, apicomplexan, chapter, factor, protozoa, treatment	Equine protozoal myeloencephalitis, horse, <i>Neospora</i> , <i>Toxoplasma</i> , <i>Sarcocystis</i>	Italy
8	Foxes	Parasite, prevalence, <i>Toxoplasma gondii</i> antibody	<i>Vulpes vulpes</i> (red fox),	Czech republic, Germany
9	Prevalence in Columbia	Presence, seroprevalence		Columbia

**Figure 2.** VOSviewer network with nine colour-coded clusters produced using VOSviewer and the Dimensions dataset containing 2935 unique publications from 1989 to 2019 inclusive (for clarity, links to the terms “Colombia” and “seroprevalencia” have been truncated at the bottom right hand corner).

2.3. SciMAT

The identification of *N. caninum* in dogs [10] and, subsequently, as a cause of abortion in cattle [2] was a significant milestone in *N. caninum* research. This is reflected in the longitudinal study performed using SciMAT and the WoS dataset (3470 publications); between 1989 and 1996 (in a dataset of $n = 15$ publications) the term “dogs” is commonly used in association with terms such as “abortion, fetuses and infection”. PCR emerges as a common technology being used in these studies. In the period of

1997–2005 ($n = 733$ publications), “abortion” emerges as the main motor theme and seroprevalence studies of herds using Enzyme-linked immunosorbent assays (ELISA) technology is recognized through association. During 2006–2012 (1172 publications), “seroprevalence” becomes the main motor theme identified by SciMAT, which is associated with words such as “risk factors, animals, goats and horses”. In the 2013–2019 ($n = 1013$) period, “infection” (which has been a feature as a secondary theme throughout the time periods) is now the major motor theme in association with most of the topics identified previously including dogs, animals, herds, risk factors, abortion, antibodies, PCR and seroprevalence (Figure 3).

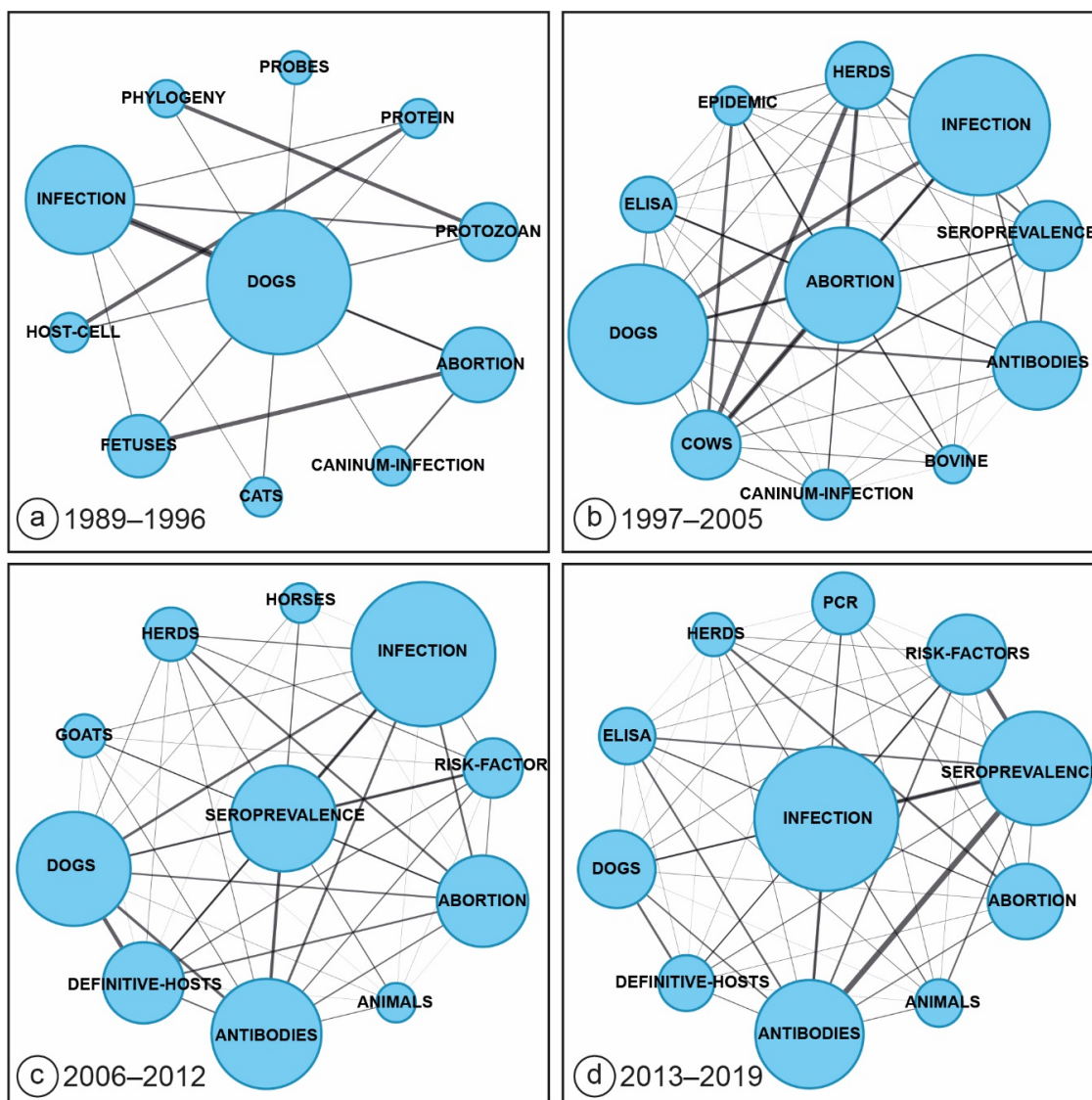


Figure 3. Main themes in *Neospora*-related publications from the Web of Science (WoS) over four time periods identified using SciMAT ((a)—1989–1996 ($n = 15$); (b)—1997–2005 ($n = 733$); (c)—2006–2012 ($n = 1172$) and (d)—2013–2019 ($n = 1013$)). The figure shows the links in terms to the four major themes identified during these time periods.

2.4. Systematic Review of Literature

The systematic literature analysis, guided by SciMAT, yielded a reduced literature field of a total of 92 scientific manuscripts meeting the citation criteria: five publications with citation ratios above 5 were included for the 1989–1996 (“dog”) time period; for the 1997–2005 (“abortion”) period,

54 publications; for the 2006–2012 period (“seroprevalence”) 17 publications, and 16 publications for the 2013–2019 (“infection”) period (Figure 4).

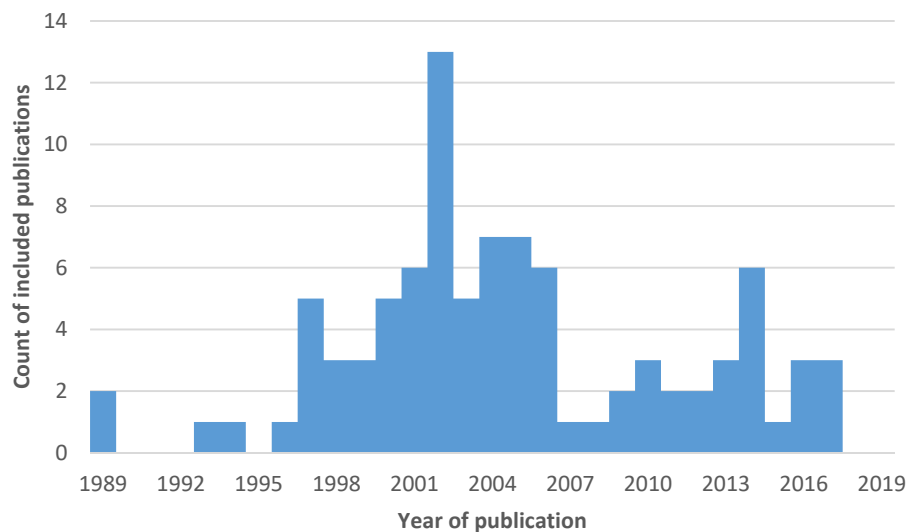


Figure 4. Frequency histogram of 92 publications derived from a systematic search of the *Neospora* literature, conducted in March 2020 in the Dimensions database for search terms: *Neospora*, dogs, abortion, seroprevalence and infection; showing peak publication activity between 1996 and 2006, with the highest number of publications in the search yielding from the year 2002.

2.4.1. Time Period 1989 to 1996 (*N. caninum* and Dog)

The five publications extracted from the ScIMAT-guided literature review yielded three publications that described the establishment, validation and use of serological techniques for the detection of *N. caninum* infection in dogs, such as Immunohistochemistry (IHC) [1], Enzyme-linked immunosorbent assays (ELISA) [18] and Indirect Fluorescent Antibody Test (IFAT) [18,19]. One study used the IFAT as a diagnostic criterion for establishing *N. caninum* infection in dogs and described the clinical aspects of a number of cases [12], while the other used IFAT to describe the prevalence of infection in a population of dogs in the United Kingdom [19].

One of the included studies also confirmed, via experimental infection that *N. caninum* can be passed from bitch to pup in utero [11].

2.4.2. Time Period 1997 to 2005 (*N. caninum* and Abortion)

This time period yielded the highest total (54) of publications from the literature review and more than 50% of the overall total included in the final literature appraisal, as well as the highest number of included publications ($n = 13$) from a single year (2002). The majority of publications in that time period focused on an understanding of the transmission of the parasite, control and its epidemiology, while a considerable number of manuscripts elaborated on diagnostic test development, validation and their application.

Varying technologies were used in establishing and validating serological tests; immunostimulating complexes (ISCOM) [20] and avidity ELISA [21], and the use of purified [22], even recombinant antigens [23], the application to different testing media, such as milk [24] and different test modalities [25], as well as adjustments to cut-off thresholds in the assays [26]. Immunoblot analysis described reaction patterns that could be used in the diagnosis [27]. Other diagnostic tools, such as histopathology, IHC and PCR were evaluated and validated [28–31]. The severity of fetal lesions contributing to abortions was also assessed [32].

Our knowledge on the transmission of the parasite in cattle populations was enhanced by an understanding of the relative importance of vertical [33–37] versus post-natal transmission [38–43],

often occurring simultaneously in the same cattle herd [44]. Based on that a better understanding of the risk factors contributing to *N. caninum* abortions was achieved [45–47], and mitigation strategies suggested, such as, in one study, the use of beef semen [48].

Two studies focused on the economic losses due to *N. caninum* infection in dairy cattle production systems [49,50], concluding that milk production was significantly reduced in herds experiencing an abortion outbreak, and putting a cost estimate on production impacts per infected 50 cow herd.

Three publications explored treatment options for *N. caninum* infection, using antiprotozoal drugs, first in vitro [51], others in a mouse model [52], ultimately in cattle [53]. The latter study showed complete resolution of an experimental infection with tachyzoites of *N. caninum*.

Characterisation of a number of isolates of *N. caninum* was undertaken, in Australia [54], Portugal [55] and isolates compared to other apicomplexans [56]. *N. caninum* isolates showed themselves to be unique to other apicomplexan parasites, with genetic markers being able to be used to differentiate between different isolates.

Studies into the immunity of *N. caninum* infection were published in that time period [57–59]. With a better understanding of the immunological consequences of infection, subsequent efforts to produce efficacious vaccines, first in a mouse model [60], then also in the target species, cattle [61] were described. This inactivated vaccine reduced abortions in cattle by about 50%.

Further seroprevalence studies added information on *N. caninum* occurrence in cattle around the world, from Iran [62] and Brazil [63], and concluded that it rarely occurred in sheep or pigs [64].

2.4.3. Time Period 2006 to 2012 (*N. caninum* and Seroprevalence)

The total of 17 publications included in this time period describe the seroprevalence of *N. caninum* infection in a number of species and countries; one study reported on the dingo (*Canis lupus dingo*), after the realisation a couple of years earlier that the dingo can also be a definitive host for *N. caninum* in Australia [14]. This study [65] found a high (27.0%) seroprevalence in wild and aboriginal dogs in indigenous communities in Australia, as well as some oocysts.

Another intermediate host was determined in a study on chickens' ability to host *N. caninum* [66], and a high rate of serological reactions was determined in ravens (*Corvus corax*) [67].

Several studies described the seroprevalence and risk factors of *N. caninum* infection in cattle populations (beef and dairy), across a number of countries and continents; such as Spain [68,69], Senegal (West-Africa) [70], North [71,72] and South America [73], as well as a multi-national study from Europe showing wide differences in individual and herd-level infection rates [74]. Risk factors repeatedly identified included the presence of dogs and the feeding of colostrum. Seroprevalence of *N. caninum*, in general, was lower in beef than in dairy cattle.

An additional study, from Brazil, established infection rates in sheep [75].

Several studies focused on *N. caninum* infection in wildlife, such as wild foxes and deer in Europe [76,77], various wildlife species in Alaska [78] or the USA [79], and zoo populations in Europe [80].

One study simulated four different control strategies for *N. caninum*, including maintaining the *status quo* (doing nothing), test and cull, treatment and vaccination [81], concluding that complete eradication was not possible if horizontal transmission of the parasite could not be curtailed.

2.4.4. Time Period 2013 to 2019 (*N. caninum* and Infection)

The 16 publications group into several topic areas; studies of the prevalence and risk factors for *N. caninum* infection for different species in different parts of the world, as varied as dogs [82], cats [83], cattle [84], small ruminants [85–87], horses [88] and wildlife [89].

More general investigations on the immune responses after an *N. caninum* infection were described in sheep as a model of infection [90], and in cattle of specific antibodies as a predictor of abortion [91], as well as the importance of the specific isolate that causes infection leading to abortion in cattle [92], and in cattle [93] and dogs [94] on Neutrophil Extracellular Traps (NET) Release.

A further publication explored calcium-dependent protein kinases as drug targets for apicomplexans, including *N. caninum* [95], another utilised an in silico approach to identify potential drug candidates against *N. caninum* [96].

2.4.5. All Time Periods

While this systematic literature search covered seminal publications that indicate, and likely influenced the direction that *N. caninum* research took over the past three decades, each of those directions is indicative of a larger body of scientific work that developed along similar lines. An author network from 1107 publications from Dimensions demonstrates clearly a central, well interlinked node of ~125 authors in the *N. caninum* scientific literature, along a (North and South) American continent–Europe centric axis, with peripherally several smaller nodes in places such as New Zealand, Australia, China and Japan contributing to the significant literature on *N. caninum* from around the world (Figure 5).

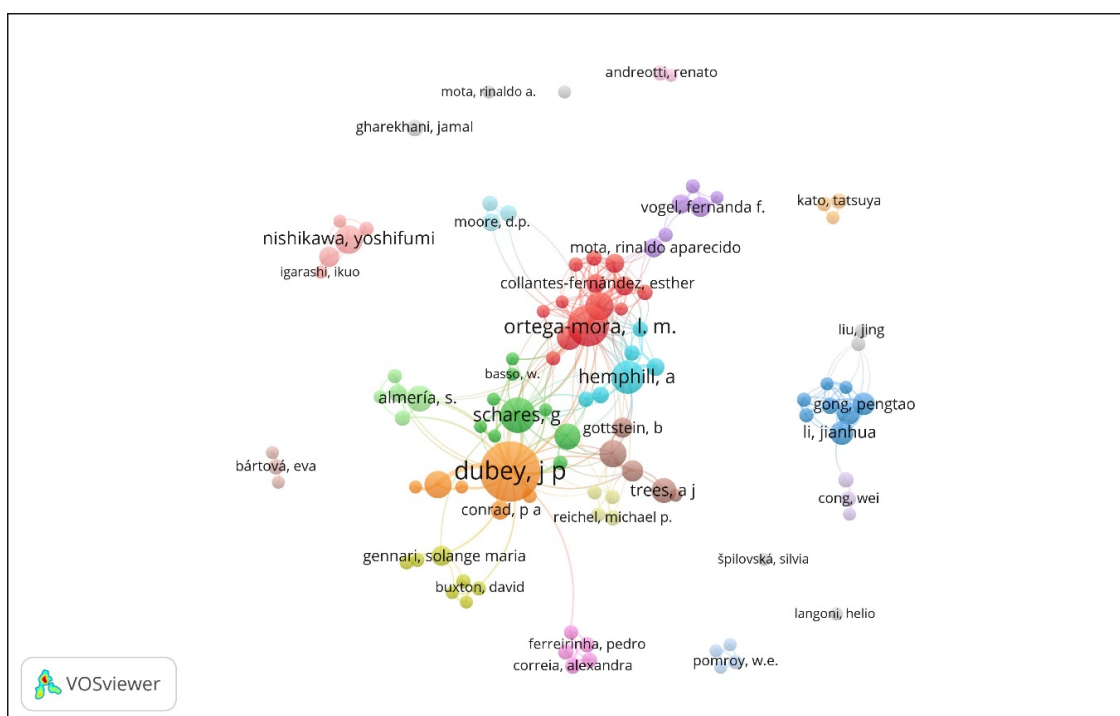


Figure 5. Author network from 1107 *N. caninum* publications from targeted SciMAT process.

3. Discussion

The scientific literature on *N. caninum* over the past 30 years developed after the initial recognition of the infection in dogs in the late 1980s. Topical modelling of the relevant literature describes *N. caninum* research over the past three decades focussed on abortions in cattle, the validation of various diagnostic tests and the application of these tests to the investigation of the epidemiology of infection, and the risk factors contributing to abortion events. VOSviewer analysis and word clusters develop similar themes around the diagnosis and epidemiology of abortions due to *N. caninum* in cattle. These advances allowed us to diagnose abortions in cattle accurately, establish that two quite different modes of transmission of the infection are observed and what risk factors might contribute to abortion (epidemic) events and should, therefore be mitigated.

The SciMAT-guided systematic literature review identified a separate guiding theme for four distinct time periods; the first time period from 1989 to 1996 focused on *N. caninum* and dogs. Diagnostic tests for *N. caninum* infection in dogs were first described and validated, and the experimental studies confirmed that infection can be vertically transmitted in dogs.

In the early parts of the subsequent time period (1997–2005), came the confirmation that dogs are one definitive host of *N. caninum* in which the sexual reproduction of the parasite is completed [10]. Other canids, such as coyotes [13] were also identified as potential definitive hosts. Further on in that period, which was also the most productive in terms of the number of highly regarded publications, the focus soon switched to cattle, with the realisation of *N. caninum* as a significant cause of abortion in that species. That time period then concentrated on diagnostic test development and validation, and their application to populations of cattle around the globe. The identification of two types of transmission modalities in cattle, vertical and post-natal, led to an increased interest in epidemiological studies, describing these two modalities, as well as risk factors for either of these two modes to be realised. The immunological events and the timing of infection driving either outcomes in cattle (abortion or congenital infection) were studied [97]. A better understanding of the underlying immunology led to a search for and application of vaccines, although early vaccines had limited efficacy in preventing abortion [61] and have subsequently been withdrawn from the market.

In the third examined time period (2006 to 2012), there was an increased interest in other possibly *N. caninum* affected species, such as wildlife, birds, small ruminant populations, as well as further research on prevalence studies around the world, enabling a better understanding of the prevalence and epidemiology (possible wildlife reservoirs, incl. the identification of the dingo in Australia as a definitive host species [14]) as well as a simulation of efficacious and economic control strategies. A better understanding of the parasite-cattle interaction led to experimental vaccines that were 100% efficacious [98].

The most recent time period (to 2019), continues to explore additional host species (wildlife, small ruminants) and explores a more sophisticated understanding of the immunology of infection and possible drug targets in cattle.

The changing themes over the time period show progress in line with the developing needs of our understanding of the parasite and its epidemiology and how research responded to these challenges. Research improved our understanding of *N. caninum*, first with the discovery of the novel parasite in dogs, then as an abortifacient in cattle, and was aided by the development of (better) diagnostic techniques and tests. Ultimately, their broader application lead to a better understanding of its epidemiology and immunology. A better understanding of the epidemiology (incidence and prevalence) also allowed a better assessment of the economic cost of the losses incurred by cattle owners and gave rise to research into vaccines to counter these losses.

The research described here has been key to our improved understanding of the diagnosis, disease ecology and epidemiology of *N. caninum* and the immune responses to its infection. Risk factor analysis has promoted improved biosecurity measures on the farm [99]; and analysis of the economic impact of *N. caninum* [4] should have provided an incentive for the funding of future research.

This next phase of research should see a substantially increased focus on drug and vaccine development [100]. Our understanding of the parasite allows us to focus efforts on efficaciously breaking the transmission cycle with appropriate vaccines [98]; future vaccines that need to be commercially acceptable.

In silico approaches to vaccine development for *N. caninum* were proposed in recent years, including those built on the reverse vaccinology pipeline *Vacceed* [101]. Other studies highlighted the need for improved genome assemblies [102] and detailed annotation of the *N. caninum* genome [103]. It is anticipated that genome assemblies from long and short read sequencing technology will lead to new advances in the application of reverse vaccinology to *N. caninum* [104] and ultimately efficacious vaccines for that parasite.

Drug development needs to focus on economically viable treatments, that are also safe. A starting point might be the re-evaluation and re-purposing of established anti-parasitic drugs, first *in-vitro* and then subsequently their evaluation in the appropriate animal models [96,105,106].

There also seems, at least occasionally, and particularly from Brazil, evidence that infection with *N. caninum* can be detected in humans, most recently *N. caninum* genetic material in the umbilical

cord bloods of a small number of fetuses. About a quarter of the cord samples tested also showed IgG antibodies to *N. caninum* in the IFAT [107]. This study also suggested that an association existed between positive sero-status and exposure to dogs. This follows on from an earlier study from that country that demonstrated a high level of sero-positivity (38%) in samples from HIV infected individuals, 18% in people with neurological symptoms but also with lower percentages (5%) from newborns [108]. Other studies had previously concluded that evidence of *N. caninum* infection was not detectable in human blood sample panels [109] or responsible for reproductive failure in humans [110]. These recent observations might warrant further investigation.

4. Materials and Methods

Selected publications were analysed in SWIFT-Review [16]; the data from a MEDLINE (through PubMed; <https://pubmed.ncbi.nlm.nih.gov/>) search with the term “*Neospora*” was exported to a file as a PMID list, which was then imported into SWIFT-Review for topic modelling. Since MEDLINE does not contain citation data, other databases were used as the source of bibliographic data.

The entire bibliometric data from a “*Neospora*” search (title and abstract, date accessed 25 March 2020) for the time period studied was exported from the Dimensions database as a csv file and any duplicates present identified and removed using SQL in the DB Browser for SQLite; the revised dataset was imported into VOSviewer [17]. A bibliometric analysis was performed based on co-occurrence of words in paper titles using binary counting; a minimum of 10 occurrences in the dataset was specified.

In order to document the evolution of research on *N. caninum*, the bibliometric data present in WoS (which including authors key words) for the topic “*Neospora*” (accessed on 25 March 2020) was obtained. This data was imported into SciMAT [111] for analyses; singular and plural versions of the same words were grouped. An analysis for co-occurrence of words over time was performed under varying parameters. The publications were arbitrarily assigned to four time periods (1989–1996, 1997–2005, 2006–2012, 2013–2019) so that changes over this chronological period could be investigated, using the workflow described elsewhere [112]. In summary, normalisation was performed using the equivalence index; the simple centers algorithm was used and the minimum network size was 3. Evolution and overlapping maps used the Jaccard and inclusion indices, respectively. Important motor-themes were identified by their location in the upper-right hand quadrant of the strategic diagram generated by SciMAT.

A systematic review of the total available English language peer-reviewed literature on *Neospora caninum* in the Dimensions database (accessed in 25 March 2020; 2933 publications) was carried out on the four main motor themes (e.g., *Neospora* AND abortion for 1997–2005) identified in SciMAT. Publications were restricted to the relevant time period related to the motor theme. Publications were assessed according to Relative Citation Ratio (RCR) for the 1989–1996 time period and Field Citation Ratio (FCR) for the other three, previously defined time periods of 1997–2005, 2006–2012 and 2013–2019. A value for the RCR or FCR of greater than 5 (as a proxy indicating peer-acknowledged quality) was used to justify inclusion of a publication for review and inclusion in this study. Reviews, editorials and Short communications were excluded from further analysis (Figure 6).

Co-authorship networks were constructed in VOSviewer using a dataset obtained from the Dimensions database. This was guided by the SciMAT outcomes and constructed for the four time periods using searches of the title and abstract for (*Neospora* AND dog, 1989–1996) plus (*Neospora* AND abortion, 1997–2005) plus (*Neospora* AND seroprevalence, 2006–2012) plus (*Neospora* AND infection, 2013–2019). This generated 1107 publications. Where multiple forms of authors names existed (e.g., Dubey, J.P., Dubey JP), they were standardised to one of the options using search/replace in Excel. Networks were constructed using fractional counting and a minimum number of publications per author of 5.

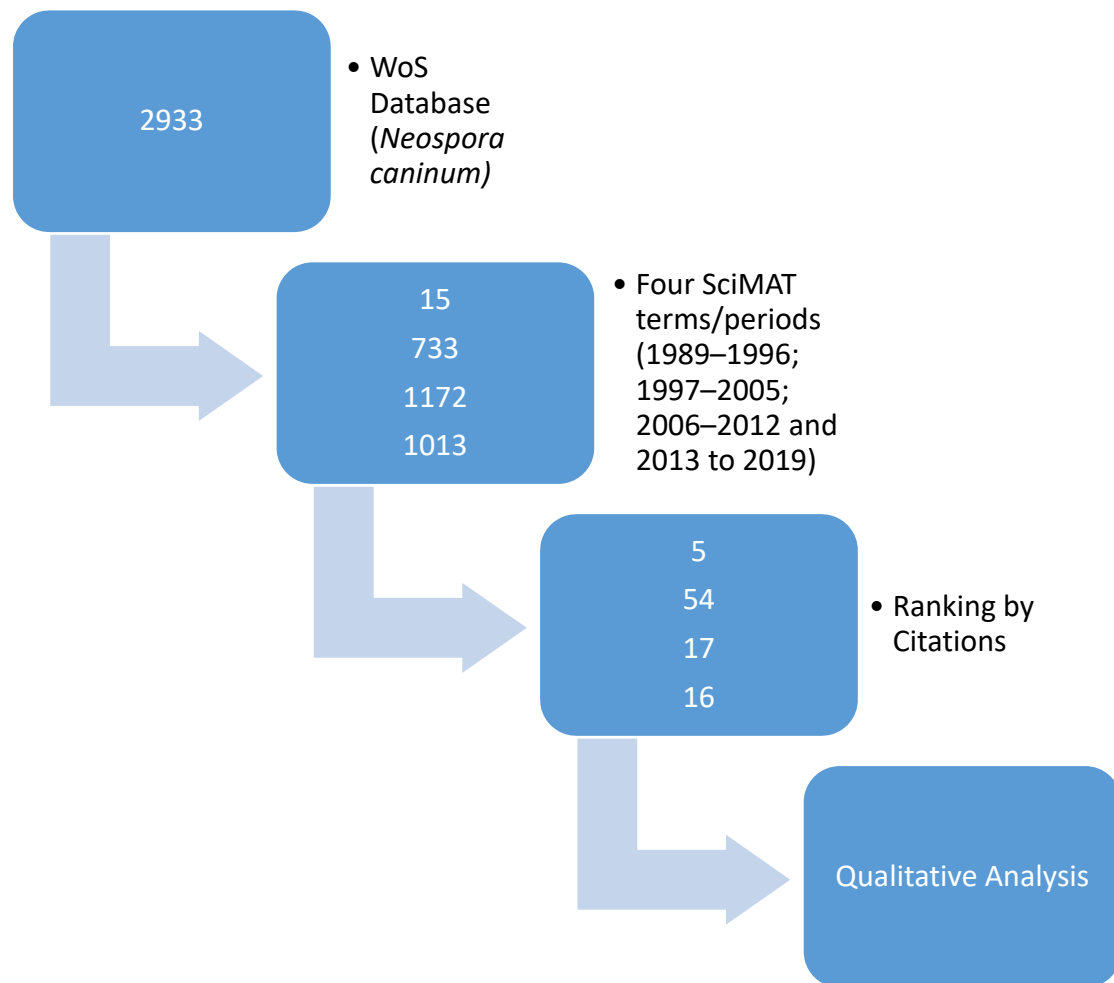


Figure 6. Graphical depiction of the systematic literature review process of the WoS database (numbers in the blue boxes represent the number of papers analysed at that step).

Author Contributions: All authors contributed equally to the conception and execution of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We are grateful to Manuel Jesus Cobo (Department of Computer Science and Engineering, Universidad de Cádiz, Spain) for his guidance with SciMAT and Bethany V Ellis for help with preparing the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lindsay, D.S.; Dubey, J.P. Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. *Am. J. Vet. Res.* **1989**, *50*, 1981–1983.
2. Thilsted, J.P.; Dubey, J.P. Neosporosis-Like Abortions in a Herd of Dairy Cattle. *J. Vet. Diagn. Investig.* **1989**, *1*, 205–209. [[CrossRef](#)]
3. Dubey, J. Congenital neosporosis in a calf. *Vet. Rec.* **1989**, *125*, 486. [[CrossRef](#)]
4. Reichel, M.P.; Ayanegui-Alcérrec, M.A.; Gondim, L.F.; Ellis, J. What is the global economic impact of *Neospora caninum* in cattle—The billion dollar question. *Int. J. Parasitol.* **2013**, *43*, 133–142. [[CrossRef](#)] [[PubMed](#)]
5. Dubey, J.P.; Hemphill, A.; Calero-Bernal, R.; Schares, G. *Neosporosis in Animals*; CRC Press: Boca Raton, FL, USA, 2017; pp. 1–529.

6. Dubey, J.P.; Lindsay, D.S. Neospora Caninum Induced Abortion in Sheep. *J. Vet. Diagn. Investig.* **1990**, *2*, 230–233. [[CrossRef](#)] [[PubMed](#)]
7. Barr, B.C.; Anderson, M.L.; Woods, L.W.; Dubey, J.P.; Conrad, P.A. Neospora-Like Protozoal Infections Associated with Abortion in Goats. *J. Vet. Diagn. Investig.* **1992**, *4*, 365–367. [[CrossRef](#)] [[PubMed](#)]
8. Bishop, S.; King, J.; Windsor, P.; Reichel, M.P.; Ellis, J.; Šlapeta, J. The first report of ovine cerebral neosporosis and evaluation of Neospora caninum prevalence in sheep in New South Wales. *Vet. Parasitol.* **2010**, *170*, 137–142. [[CrossRef](#)]
9. Reichel, M.P.; McAllister, M.; Nasir, A.; Moore, D. A review of Neospora caninum in water buffalo (*Bubalus bubalis*). *Vet. Parasitol.* **2015**, *212*, 75–79. [[CrossRef](#)] [[PubMed](#)]
10. McAllister, M.; Dubey, J.P.; Lindsay, D.S.; Jolley, W.R.; Wills, R.A.; McGuire, A.M. Dogs are definitive hosts of Neospora caninum. *Int. J. Parasitol.* **1998**, *28*, 1473–1478. [[CrossRef](#)]
11. Dubey, J.P.; Lindsay, D.S. Transplacental Neospora caninum infection in dogs. *Am. J. Vet. Res.* **1989**, *50*, 1578–1579.
12. Barber, J.S.; Trees, A.J. Clinical aspects of 27 cases of neosporosis in dogs. *Vet. Rec.* **1996**, *139*, 439–443. [[CrossRef](#)] [[PubMed](#)]
13. Gondim, L.F.; McAllister, M.; Pitt, W.C.; Zemlicka, D.E. Coyotes (*Canis latrans*) are definitive hosts of Neospora caninum. *Int. J. Parasitol.* **2004**, *34*, 159–161. [[CrossRef](#)]
14. King, J.S.; Šlapeta, J.; Jenkins, D.J.; Al-Qassab, S.E.; Ellis, J.; Windsor, P. Australian dingoes are definitive hosts of Neospora caninum. *Int. J. Parasitol.* **2010**, *40*, 945–950. [[CrossRef](#)]
15. Dubey, J.; Buxton, D.; Wouda, W. Pathogenesis of Bovine Neosporosis. *J. Comp. Pathol.* **2006**, *134*, 267–289. [[CrossRef](#)] [[PubMed](#)]
16. Howard, B.E.; Phillips, J.; Miller, K.; Tandon, A.; Mav, D.; Shah, M.R.; Holmgren, S.; Pelch, K.E.; Walker, V.; Rooney, A.A.; et al. SWIFT-Review: A text-mining workbench for systematic review. *Syst. Rev.* **2016**, *5*, 87. [[CrossRef](#)] [[PubMed](#)]
17. Van Eck, N.J.; Waltman, L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* **2009**, *84*, 523–538. [[CrossRef](#)]
18. Björkman, C.; Lundén, A.; Holmdahl, J.; Barber, J.; Trees, A.J.; Uggla, A. Neospora caninum in dogs: Detection of antibodies by ELISA using an iscom antigen. *Parasite Immunol.* **1994**, *16*, 643–648. [[CrossRef](#)]
19. Trees, A.; Guy, F.; Tennant, B.; Balfour, A.; Dubey, J. Prevalence of antibodies to Neospora caninum in a population of urban dogs in England. *Vet. Rec.* **1993**, *132*, 125–126. [[CrossRef](#)] [[PubMed](#)]
20. Frössling, J.; Bonnett, B.; Lindberg, A.; Björkman, C. Validation of a Neospora caninum iscom ELISA without a gold standard. *Prev. Vet. Med.* **2003**, *57*, 141–153. [[CrossRef](#)]
21. Björkman, C.; McAllister, M.; Frössling, J.; Näslund, K.; Leung, F.; Uggla, A. Application of the Neospora caninum IgG avidity ELISA in assessment of chronic reproductive losses after an outbreak of neosporosis in a herd of beef cattle. *J. Vet. Diagn. Investig.* **2003**, *15*, 3–7. [[CrossRef](#)] [[PubMed](#)]
22. Schares, G.; Rauser, M.; Söndgen, P.; Rehberg, P.; Bärwald, A.; Dubey, J.; Edelhofer, R.; Conraths, F.J. Use of purified tachyzoite surface antigen p38 in an ELISA to diagnose bovine neosporosis. *Int. J. Parasitol.* **2000**, *30*, 1123–1130. [[CrossRef](#)]
23. Chahan, B.; Gaturaga, I.; Huang, X.; Liao, M.; Fukumoto, S.; Hirata, H.; Nishikawa, Y.; Suzuki, H.; Sugimoto, C.; Nagasawa, H.; et al. Serodiagnosis of Neospora caninum infection in cattle by enzyme-linked immunosorbent assay with recombinant truncated NcSAG1. *Vet. Parasitol.* **2003**, *118*, 177–185. [[CrossRef](#)] [[PubMed](#)]
24. Schares, G.; Bärwald, A.; Staubach, C.; Wurm, R.; Rauser, M.; Conraths, F.J.; Schroeder, C. Adaptation of a commercial ELISA for the detection of antibodies against Neospora caninum in bovine milk. *Vet. Parasitol.* **2004**, *120*, 55–63. [[CrossRef](#)] [[PubMed](#)]
25. Romand, S.; Thulliez, P.; Dubey, J. Direct agglutination test for serologic diagnosis of Neospora caninum infection. *Parasitol. Res.* **1998**, *84*, 50–53. [[CrossRef](#)]
26. Álvarez-García, G.; Collantes-Fernandez, E.; Costas, E.; Rebordosa, X.; Ortega-Mora, L.M. Influence of age and purpose for testing on the cut-off selection of serological methods in bovine neosporosis. *Vet. Res.* **2003**, *34*, 341–352. [[CrossRef](#)]
27. Álvarez-García, G.; Pereira-Bueno, J.; Gómez-Bautista, M.; Ortega-Mora, L.M. Pattern of recognition of Neospora caninum tachyzoite antigens by naturally infected pregnant cattle and aborted fetuses. *Vet. Parasitol.* **2002**, *107*, 15–27. [[CrossRef](#)]

28. Van Maanen, C.; Wouda, W.; Schares, G.; Von Blumröder, D.; Conraths, F.J.; Norton, R.; Williams, D.; Esteban-Redondo, I.; Innes, E.A.; Mattsson, J.; et al. An interlaboratory comparison of immunohistochemistry and PCR methods for detection of *Neospora caninum* in bovine foetal tissues. *Vet. Parasitol.* **2004**, *126*, 351–364. [[CrossRef](#)]
29. Collantes-Fernández, E.; Zaballos, Á.; Álvarez-García, G.; Ortega-Mora, L.M. Quantitative Detection of *Neospora caninum* in Bovine Aborted Fetuses and Experimentally Infected Mice by Real-Time PCR. *J. Clin. Microbiol.* **2002**, *40*, 1194–1198. [[CrossRef](#)]
30. Sager, H.; Fischer, I.; Furrer, K.; Strasser, M.; Waldvogel, A.; Boerlin, P.; Audigé, L.; Gottstein, B. A Swiss case-control study to assess *Neospora caninum*-associated bovine abortions by PCR, histopathology and serology. *Vet. Parasitol.* **2001**, *102*, 1–15. [[CrossRef](#)]
31. Morales-Salinas, E.; Trigo, F.; Ibarra, F.; Puente, E.; Santacruz, M. Neosporosis in Mexican Dairy Herds: Lesions and Immunohistochemical Detection of *Neospora caninum* in Fetuses. *J. Comp. Pathol.* **2001**, *125*, 58–63. [[CrossRef](#)]
32. Wouda, W.; Moen, A.R.; Visser, I.J.R.; Van Knapen, F. Bovine fetal neosporosis: A comparison of epizootic and sporadic abortion cases and different age classes with regard to lesion severity and immunohistochemical identification of organisms in brain, heart, and liver. *J. Vet. Diagn. Investig.* **1997**, *9*, 180–185. [[CrossRef](#)] [[PubMed](#)]
33. Hall, C.; Reichel, M.P.; Ellis, J. *Neospora* abortions in dairy cattle: Diagnosis, mode of transmission and control. *Vet. Parasitol.* **2005**, *128*, 231–241. [[CrossRef](#)]
34. Davison, H.C.; Otter, A.; Trees, A. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infections in dairy cattle. *Int. J. Parasitol.* **1999**, *29*, 1683–1689. [[CrossRef](#)]
35. Schares, G.; Peters, M.; Wurm, R.; Bärwald, A.; Conraths, F.J. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. *Vet. Parasitol.* **1998**, *80*, 87–98. [[CrossRef](#)]
36. Thurmond, M.C.; Hietala, S. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. *Am. J. Vet. Res.* **1997**, *58*, 1381–1385.
37. Pare, J.; Thurmond, M.C.; Hietala, S.K. *Neospora caninum* Antibodies in Cows during Pregnancy as a Predictor of Congenital Infection and Abortion. *J. Parasitol.* **1997**, *83*, 82–87. [[CrossRef](#)]
38. Pfeiffer, D.U.; Williamson, N.; Reichel, M.P.; Wichtel, J.; Teague, W. A longitudinal study of *Neospora caninum* infection on a dairy farm in New Zealand. *Prev. Vet. Med.* **2002**, *54*, 11–24. [[CrossRef](#)]
39. Dijkstra, T.; Barkema, H.W.; Hesselink, J.; Wouda, W. Point source exposure of cattle to *Neospora caninum* consistent with periods of common housing and feeding and related to the introduction of a dog. *Vet. Parasitol.* **2002**, *105*, 89–98. [[CrossRef](#)]
40. Dijkstra, T.; Barkema, H.W.; Eysker, M.; Wouda, W. Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int. J. Parasitol.* **2001**, *31*, 209–215. [[CrossRef](#)]
41. McAllister, M.M.; Björkman, C.; Anderson-Sprecher, R.; Rogers, D.G. Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. *J. Am. Vet. Med. Assoc.* **2000**, *217*, 881–887. [[CrossRef](#)]
42. Atkinson, R.; Cook, R.; Reddacliff, L.; Rothwell, J.; Broady, K.; Harper, P.; Ellis, J.; Ellis, J. Seroprevalence of *Neospora caninum* infection following an abortion outbreak in a dairy cattle herd. *Aust. Vet. J.* **2000**, *78*, 262–266. [[CrossRef](#)]
43. Thurmond, M.C.; Hietala, S.K.; Blanchard, P.C. Herd-Based Diagnosis of *Neospora Caninum*-Induced Endemic and Epidemic Abortion in Cows and Evidence for Congenital and Postnatal Transmission. *J. Vet. Diagn. Investig.* **1997**, *9*, 44–49. [[CrossRef](#)]
44. Bergeron, N.; Fecteau, G.; Paré, J.; Martineau, R.; Villeneuve, A. Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Québec. *Can. Vet. J.* **2000**, *41*, 464–467.
45. Moen, A.; Wouda, W.; Mul, M.; Graat, E.; Van Werven, T. Increased risk of abortion following neospora caninum abortion outbreaks: A retrospective and prospective cohort study in four dairy herds. *Theriogenology* **1998**, *49*, 1301–1309. [[CrossRef](#)]
46. Bartels, C.; Wouda, W.; Schukken, Y. Risk factors for neospora caninum-associated abortion storms in dairy herds in The Netherlands (1995 to 1997). *Theriogenology* **1999**, *52*, 247–257. [[CrossRef](#)]

47. Hobson, J.; Duffield, T.; Kelton, D.; Lissemore, K.; Hietala, S.; Leslie, K.; McEwen, B.; Peregrine, A. Risk factors associated with *Neospora caninum* abortion in Ontario Holstein dairy herds. *Vet. Parasitol.* **2005**, *127*, 177–188. [[CrossRef](#)]
48. Lopez-Gatius, F.; Santolaria, P.; Yániz, J.L.; Garbayo, J.M.; Almeria, S. The Use of Beef Bull Semen Reduced the Risk of Abortion in *Neospora*-seropositive Dairy Cows. *J. Vet. Med. Ser. B* **2005**, *52*, 88–92. [[CrossRef](#)]
49. Chi, J.; VanLeeuwen, J.A.; Weersink, A.; Keefe, G.P. Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies paratuberculosis, and *Neospora caninum*. *Prev. Vet. Med.* **2002**, *55*, 137–153. [[CrossRef](#)]
50. Hobson, J.C.; Duffield, T.F.; Kelton, D.; Lissemore, K.; Hietala, S.K.; Leslie, K.E.; McEwen, B.; Cramer, G.; Peregrine, A.S. *Neospora caninum* serostatus and milk production of Holstein cattle. *J. Am. Vet. Med. Assoc.* **2002**, *221*, 1160–1164. [[CrossRef](#)]
51. Kim, J.T.; Park, J.Y.; Seo, H.S.; Oh, H.G.; Noh, J.W.; Kim, J.H.; Kim, D.Y.; Youn, H.J. In vitro antiprotozoal effects of artemisinin on *Neospora caninum*. *Vet. Parasitol.* **2002**, *103*, 53–63. [[CrossRef](#)]
52. Gottstein, B.; Razmi, G.R.; Ammann, P.; Sager, H.; Müller, N. Toltrazuril treatment to control diaplacental *Neospora caninum* transmission in experimentally infected pregnant mice. *Parasitology* **2005**, *130*, 41–48. [[CrossRef](#)]
53. Kritzner, S.; Sager, H.; Blum, J.; Krebber, R.; Greif, G.; Gottstein, B. An explorative study to assess the efficacy of Toltrazuril-sulfone (Ponazuril) in calves experimentally infected with *Neospora caninum*. *Ann. Clin. Microbiol. Antimicrob.* **2002**, *1*, 4. [[CrossRef](#)]
54. Miller, C.; Quinn, H.; Windsor, P.; Ellis, J.; Ellis, J. Characterisation of the first Australian isolate of *Neospora caninum* from cattle. *Aust. Vet. J.* **2002**, *80*, 620–625. [[CrossRef](#)]
55. Basso, W.; Herrmann, D.; Conraths, F.J.; Pantchev, N.; Vrhovec, M.G.; Schares, G. First isolation of *Neospora caninum* from the faeces of a dog from Portugal. *Vet. Parasitol.* **2009**, *159*, 162–166. [[CrossRef](#)]
56. Schock, A.; Innes, E.A.; Yamane, I.; Latham, S.M.; Wastling, J.M. Genetic and biological diversity among isolates of *Neospora caninum*. *Parasitology* **2001**, *123*, 13–23. [[CrossRef](#)]
57. Williams, D.J.; Guy, C.S.; Smith, R.; Guy, F.; McGarry, J.W.; McKay, J.S.; Trees, A.J. First demonstration of protective immunity against foetopathy in cattle with latent *Neospora caninum* infection. *Int. J. Parasitol.* **2003**, *33*, 1059–1065. [[CrossRef](#)]
58. Williams, D.J.L.; Guy, C.S.; McGarry, J.W.; Guy, F.; Tasker, L.; Smith, R.F.; MacEachern, K.; Cripps, P.J.; Kelly, D.F.; Trees, A. *Neospora caninum*-associated abortion in cattle: The time of experimentally-induced parasitaemia during gestation determines foetal survival. *Parasitology* **2000**, *121*, 347–358. [[CrossRef](#)]
59. De Marez, T.; Liddell, S.; Dubey, J.P.; Jenkins, M.C.; Gasbarre, L. Oral infection of calves with *Neospora caninum* oocysts from dogs: Humoral and cellular immune responses. *Int. J. Parasitol.* **1999**, *29*, 1647–1657. [[CrossRef](#)]
60. Nishikawa, Y.; Xuan, X.; Nagasawa, H.; Igarashi, I.; Fujisaki, K.; Otsuka, H.; Mikami, T. Prevention of vertical transmission of *Neospora caninum* in BALB/c mice by recombinant vaccinia virus carrying NcSRS2 gene. *Vaccine* **2001**, *19*, 1710–1716. [[CrossRef](#)]
61. Zúñiga, J.J.R.; Perez, E.; Frankena, K. Effect of a killed whole *Neospora caninum* tachyzoite vaccine on the crude abortion rate of Costa Rican dairy cows under field conditions. *Vet. Parasitol.* **2004**, *123*, 149–159. [[CrossRef](#)]
62. Sadrebazzaz, A.; Haddadzadeh, H.; Esmailnia, K.; Habibi, G.; Vojgani, M.; Hashemifesharaki, R. Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Vet. Parasitol.* **2004**, *124*, 201–204. [[CrossRef](#)]
63. Corbellini, L.G.; Driemeier, D.; Cruz, C.F.E.; Gondim, L.F.; Wald, V. Neosporosis as a cause of abortion in dairy cattle in Rio Grande do Sul, southern Brazil. *Vet. Parasitol.* **2002**, *103*, 195–202. [[CrossRef](#)]
64. Helmick, B.; Otter, A.; McGarry, J.; Buxton, D. Serological investigation of aborted sheep and pigs for infection by *Neospora caninum*. *Res. Vet. Sci.* **2002**, *73*, 187–189. [[CrossRef](#)]
65. King, J.S.; Brown, G.K.; Jenkins, D.J.; Ellis, J.; Fleming, P.J.S.; Windsor, P.; Šlapeta, J. Oocysts and high seroprevalence of *Neospora caninum* in dogs living in remote Aboriginal communities and wild dogs in Australia. *Vet. Parasitol.* **2012**, *187*, 85–92. [[CrossRef](#)]
66. Costa, K.; Santos, S.; Uzeda, R.; Pinheiro, A.M.; Almeida, M.; Araujo, F.; McAllister, M.; Gondim, L.F. Chickens (*Gallus domesticus*) are natural intermediate hosts of *Neospora caninum*. *Int. J. Parasitol.* **2008**, *38*, 157–159. [[CrossRef](#)]

67. Molina-López, R.; Cabezón, O.; Pabón, M.; Darwich, L.; Obón, E.; Lopez-Gatius, F.; Dubey, J.; Almeria, S. High seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in the Common raven (*Corvus corax*) in the Northeast of Spain. *Res. Vet. Sci.* **2012**, *93*, 300–302. [[CrossRef](#)]
68. Eiras, C.; Arnaiz, I.; Álvarez-García, G.; Ortega-Mora, L.M.; Sanjuán, M.; Yus, E.; Diéguez, F.J. *Neospora caninum* seroprevalence in dairy and beef cattle from the northwest region of Spain, Galicia. *Prev. Vet. Med.* **2011**, *98*, 128–132. [[CrossRef](#)]
69. Panadero, R.; Panceira, A.; López, C.; Vázquez, L.; Paz, A.; Diaz, P.; DaCal, V.; Cienfuegos, S.; Fernández, P.D.; Lago, N.; et al. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). *Res. Vet. Sci.* **2010**, *88*, 111–115. [[CrossRef](#)]
70. Kamga-Waladjo, A.R.; Gbati, O.B.; Kone, P.; Lapo, R.A.; Chatagnon, G.; Bakou, S.N.; Pangu, L.J.; Diop, P.E.H.; Akakpo, J.A.; Tainturier, D. Seroprevalence of *Neospora caninum* antibodies and its consequences for reproductive parameters in dairy cows from Dakar–Senegal, West Africa. *Trop. Anim. Health Prod.* **2009**, *42*, 953–959. [[CrossRef](#)]
71. Scott, H.M.; Sorensen, O.; Wu, J.T.; Chow, E.Y.; Manninen, K.; VanLeeuwen, J.A. Seroprevalence of *Mycobacterium avium* subspecies paratuberculosis, *Neospora caninum*, Bovine leukemia virus, and Bovine viral diarrhoea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity. *Can. Vet. J.* **2006**, *47*, 981–991.
72. VanLeeuwen, J.; Tiwari, A.; Plaizier, J.C.; Whiting, T.L. Seroprevalences of antibodies against bovine leukemia virus, bovine viral diarrhoea virus, *Mycobacterium avium* subspecies paratuberculosis, and *Neospora caninum* in beef and dairy cattle in Manitoba. *Can. Vet. J.* **2006**, *47*, 783–786.
73. Corbellini, L.G.; Smith, D.R.; Pescador, C.A.; Schmitz, M.; Corrêa, A.; Steffen, D.J.; Driemeier, D. Herd-level risk factors for *Neospora caninum* seroprevalence in dairy farms in southern Brazil. *Prev. Vet. Med.* **2006**, *74*, 130–141. [[CrossRef](#)]
74. Bartels, C.; Arnaiz-Seco, J.; Ruiz-Santa-Quitera, A.; Björkman, C.; Frössling, J.; Von Blumröder, D.; Conraths, F.J.; Schares, G.; Van Maanen, C.; Wouda, W.; et al. Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, The Netherlands, Spain and Sweden. *Vet. Parasitol.* **2006**, *137*, 17–27. [[CrossRef](#)]
75. Ueno, T.E.H.; Gonçalves, V.S.P.; Heinemann, M.B.; Dilli, T.L.B.; Akimoto, B.M.; De Souza, S.L.P.; Gennari, S.M.; Soares, R.M. Prevalence of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Federal District, central region of Brazil. *Trop. Anim. Health Prod.* **2008**, *41*, 547–552. [[CrossRef](#)]
76. De Craeye, S.; Speybroeck, N.; Ajzenberg, D.; Dardé, M.L.; Collinet, F.; Tavernier, P.; Van Gucht, S.; Dorny, P.; Dierick, K. *Toxoplasma gondii* and *Neospora caninum* in wildlife: Common parasites in Belgian foxes and Cervidae? *Vet. Parasitol.* **2011**, *178*, 64–69. [[CrossRef](#)]
77. Almeria, S.; Vidal, D.; Ferrer, D.; Pabón, M.; De Mera, I.G.F.; Ruiz-Fons, J.F.; Alzaga, V.; Marco, I.; Calvete, C.; Lavín, S.; et al. Seroprevalence of *Neospora caninum* in non-carnivorous wildlife from Spain. *Vet. Parasitol.* **2007**, *143*, 21–28. [[CrossRef](#)]
78. Stieve, E.; Beckmen, K.; Kania, S.A.; Widner, A.; Patton, S. *Neospora caninum* and *Toxoplasma gondii* antibody prevalence in Alaska wildlife. *J. Wildl. Dis.* **2010**, *46*, 348–355. [[CrossRef](#)]
79. Almborg, E.S.; Mech, L.D.; Smith, D.W.; Sheldon, J.W.; Crabtree, R.L. A Serological Survey of Infectious Disease in Yellowstone National Park's Canid Community. *PLoS ONE* **2009**, *4*, e7042. [[CrossRef](#)]
80. Sedlák, K.; Bártová, E. Seroprevalences of antibodies to *Neospora caninum* and *Toxoplasma gondii* in zoo animals. *Vet. Parasitol.* **2006**, *136*, 223–231. [[CrossRef](#)]
81. Häslér, B.; Stärk, K.D.C.; Sager, H.; Gottstein, B.; Reist, M. Simulating the impact of four control strategies on the population dynamics of *Neospora caninum* infection in Swiss dairy cattle. *Prev. Vet. Med.* **2006**, *77*, 254–283. [[CrossRef](#)]
82. Langoni, H.; Fornazari, F.; Da Silva, R.C.; Monti, E.T.; Villa, F.B. Prevalence of antibodies against *Toxoplasma gondii* and *Neospora caninum* in dogs. *Braz. J. Microbiol.* **2014**, *44*, 1327–1330. [[CrossRef](#)]
83. Silaghi, C.; Knaus, M.; Rapti, D.; Kusi, I.; Shukullari, E.; Hamel, D.; Pfister, K.; Rehbein, S. Survey of *Toxoplasma gondii* and *Neospora caninum*, haemotropic mycoplasmas and other arthropod-borne pathogens in cats from Albania. *Parasites Vectors* **2014**, *7*, 62. [[CrossRef](#)]
84. Bruhn, F.R.P.; Daher, D.O.; Lopes, E.; Barbieri, J.M.; Da Rocha, C.M.B.M.; Guimarães, A.M. Factors associated with seroprevalence of *Neospora caninum* in dairy cattle in southeastern Brazil. *Trop. Anim. Health Prod.* **2012**, *45*, 1093–1098. [[CrossRef](#)]

85. Unzaga, J.; Moré, G.; Bacigalupe, D.; Rambeaud, M.; Pardini, L.; Dellarupe, A.; De Felice, L.; Gos, M.; Venturini, M. *Toxoplasma gondii* and *Neospora caninum* infections in goat abortions from Argentina. *Parasitol. Int.* **2014**, *63*, 865–867. [[CrossRef](#)]
86. González-Warleta, M.; Castro-Hermida, J.A.; Regidor-Cerrillo, J.; Benavides, J.; Álvarez-García, G.; Fuertes, M.; Ortega-Mora, L.M.; Mezo, M. *Neospora caninum* infection as a cause of reproductive failure in a sheep flock. *Vet. Res.* **2014**, *45*, 88. [[CrossRef](#)]
87. Liu, Z.K.; Li, J.Y.; Pan, H. Seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in China. *Prev. Vet. Med.* **2015**, *118*, 488–492. [[CrossRef](#)]
88. Ribeiro, M.J.M.; Rosa, M.H.F.; Bruhn, F.R.P.; Garcia, A.D.M.; Rocha, C.M.B.M.; Guimarães, A.M. Seroepidemiology of *Sarcocystis neurona*, *Toxoplasma gondii* and *Neospora* spp. among horses in the south of the state of Minas Gerais, Brazil. *Rev. Bras. Parasitol. Vet.* **2016**, *25*, 142–150. [[CrossRef](#)]
89. Lempp, C.; Jungwirth, N.; Grilo, M.L.; Reckendorf, A.; Ulrich, A.; Van Neer, A.; Bodewes, R.; Pfankuche, V.M.; Bauer, C.; Osterhaus, A.D.M.E.; et al. Pathological findings in the red fox (*Vulpes vulpes*), stone marten (*Martes foina*) and raccoon dog (*Nyctereutes procyonoides*), with special emphasis on infectious and zoonotic agents in Northern Germany. *PLoS ONE* **2017**, *12*, e0175469. [[CrossRef](#)]
90. Arranz-Solís, D.; Benavides, J.; Regidor-Cerrillo, J.; Horcajo, P.; Castaño, P.; Ferreras, M.; Jiménez-Pelayo, L.; Collantes-Fernandez, E.; Ferre, I.; Hemphill, A.; et al. Systemic and local immune responses in sheep after *Neospora caninum* experimental infection at early, mid and late gestation. *Vet. Res.* **2016**, *47*, 2. [[CrossRef](#)]
91. Garcia-Ispierto, I.; Almeria, S.; Serrano-Pérez, B.; De Sousa, N.; Beckers, J.; Lopez-Gatius, F. Plasma Concentrations of Pregnancy-Associated Glycoproteins Measured Using Anti-Bovine PAG-2 Antibodies on Day 120 of Gestation Predict Abortion in Dairy Cows Naturally Infected with *Neospora caninum*. *Reprod. Domest. Anim.* **2012**, *48*, 613–618. [[CrossRef](#)]
92. Regidor-Cerrillo, J.; Arranz-Solís, D.; Benavides, J.; Gómez-Bautista, M.; Castro-Hermida, J.A.; Mezo, M.; Perez, V.; Ortega-Mora, L.M.; González-Warleta, M. *Neospora caninum* infection during early pregnancy in cattle: How the isolate influences infection dynamics, clinical outcome and peripheral and local immune responses. *Vet. Res.* **2014**, *45*, 10. [[CrossRef](#)] [[PubMed](#)]
93. Villagra-Blanco, R.; Silva, L.M.R.; Muñoz-Caro, T.; Yang, Z.; Li, J.; Gärtner, U.; Taubert, A.; Zhang, X.; Hermosilla, C. Bovine Polymorphonuclear Neutrophils Cast Neutrophil Extracellular Traps against the Abortive Parasite *Neospora caninum*. *Front. Immunol.* **2017**, *8*, 606. [[CrossRef](#)] [[PubMed](#)]
94. Wei, Z.; Hermosilla, C.; Taubert, A.; He, X.; Wang, X.; Gong, P.; Li, J.; Yang, Z.; Zhang, X. Canine Neutrophil Extracellular Traps Release Induced by the Apicomplexan Parasite *Neospora caninum* In Vitro. *Front. Immunol.* **2016**, *7*, 436. [[CrossRef](#)]
95. Keyloun, K.R.; Reid, M.C.; Choi, R.; Song, Y.; Fox, A.M.W.; Hillesland, H.K.; Zhang, Z.; Vidadala, R.; Merritt, E.A.; Lau, A.; et al. The gatekeeper residue and beyond: Homologous calcium-dependent protein kinases as drug development targets for veterinarian Apicomplexa parasites. *Parasitology* **2014**, *141*, 1499–1509. [[CrossRef](#)]
96. Müller, J.; Aguado, A.; Laleu, B.; Balmer, V.; Ritler, D.; Hemphill, A. In vitro screening of the open source Pathogen Box identifies novel compounds with profound activities against *Neospora caninum*. *Int. J. Parasitol.* **2017**, *47*, 801–809. [[CrossRef](#)]
97. Williams, D.J.L.; Trees, A.J. Review Article. Protecting babies: Vaccine strategies to prevent foetopathy in *Neospora caninum*-infected cattle. *Parasite Immunol.* **2006**, *28*, 61–67. [[CrossRef](#)]
98. Williams, D.J.L.; Guy, C.S.; Smith, R.; Ellis, J.; Björkman, C.; Reichel, M.P.; Trees, A.J. Immunization of Cattle with Live Tachyzoites of *Neospora caninum* Confers Protection against Fetal Death. *Infect. Immun.* **2006**, *75*, 1343–1348. [[CrossRef](#)]
99. McAllister, M. Diagnosis and Control of Bovine Neosporosis. *Vet. Clin. N. Am. Food Anim. Pract.* **2016**, *32*, 443–463. [[CrossRef](#)]
100. Reichel, M.P.; Moore, D.; Hemphill, A.; Ortega-Mora, L.M.; Dubey, J.; Ellis, J. A live vaccine against *Neospora caninum* abortions in cattle. *Vaccine* **2015**, *33*, 1299–1301. [[CrossRef](#)]
101. Goodswen, S.J.; Kennedy, P.; Ellis, J. Vacceed: A high-throughput in silico vaccine candidate discovery pipeline for eukaryotic pathogens based on reverse vaccinology. *Bioinformatics* **2014**, *30*, 2381–2383. [[CrossRef](#)]
102. Calarco, L.; Barratt, J.; Ellis, J. Genome Wide Identification of Mutational Hotspots in the Apicomplexan Parasite *Neospora caninum* and the Implications for Virulence. *Genome Biol. Evol.* **2018**, *10*, 2417–2431. [[CrossRef](#)] [[PubMed](#)]

103. Calarco, L.; Ellis, J. Annotating the ‘hypothetical’ in hypothetical proteins: In-silico analysis of uncharacterised proteins for the Apicomplexan parasite, *Neospora caninum*. *Vet. Parasitol.* **2019**, *265*, 29–37. [[CrossRef](#)] [[PubMed](#)]
104. Berna, L.; Marquez, P.; Cabrera, A.; Greif, G.; Francia, M.E.; Robello, C. Reevaluation of the *Toxoplasma gondii* and *Neospora caninum* genomes reveals misassembly, karyotype differences and chromosomal rearrangements. *bioRxiv* **2020**. [[CrossRef](#)]
105. Müller, J.; Aguado-Martínez, A.; Manser, V.; Wong, H.N.; Haynes, R.K.; Hemphill, A. Repurposing of antiparasitic drugs: The hydroxy-naphthoquinone buparvaquone inhibits vertical transmission in the pregnant neosporosis mouse model. *Vet. Res.* **2016**, *47*, 32. [[CrossRef](#)]
106. Hemphill, A.; Aguado-Martínez, A.; Müller, J. Approaches for the vaccination and treatment of *Neospora caninum* infections in mice and ruminant models. *Parasitology* **2015**, *143*, 245–259. [[CrossRef](#)]
107. Duarte, P.O.; Oshiro, L.M.; Zimmermann, N.P.; Csordas, B.G.; Dourado, D.M.; Barros, J.C.; Andreotti, R. Serological and molecular detection of *Neospora caninum* and *Toxoplasma gondii* in human umbilical cord blood and placental tissue samples. *Sci. Rep.* **2020**, *10*, 9043. [[CrossRef](#)]
108. Lobato, J.; Silva, D.A.O.; Mineo, T.W.P.; Amaral, J.D.H.F.; Segundo, G.R.S.; Costa-Cruz, J.M.; Ferreira, M.S.; Borges, A.S.; Mineo, J.R. Detection of Immunoglobulin G Antibodies to *Neospora caninum* in Humans: High Seropositivity Rates in Patients Who Are Infected by Human Immunodeficiency Virus or Have Neurological Disorders. *Clin. Vaccine Immunol.* **2006**, *13*, 84–89. [[CrossRef](#)]
109. McCann, C.M.; Vyse, A.J.; Salmon, R.L.; Thomas, D.; Williams, D.J.; McGarry, J.W.; Pebody, R.; Trees, A. Lack of Serologic Evidence of *Neospora caninum* in Humans, England. *Emerg. Infect. Dis.* **2008**, *14*, 978–980. [[CrossRef](#)] [[PubMed](#)]
110. Petersen, E.; Lebech, M.; Jensen, L.; Lind, P.; Rask, M.; Bagger, P.; Björkman, C.; Ugglå, A. *Neospora caninum* infection and repeated abortions in humans. *Emerg. Infect. Dis.* **1999**, *5*, 278–280. [[CrossRef](#)] [[PubMed](#)]
111. Cobo, M.J.; Herrera-Viedma, E.; Herrera, F.; López-Herrera, A. SciMAT: A new science mapping analysis software tool. *J. Am. Soc. Inf. Sci. Technol.* **2012**, *63*, 1609–1630. [[CrossRef](#)]
112. Cobo, M.-J.; López-Herrera, A.G.; Herrera-Viedma, E.; Herrera, F. An approach for detecting, quantifying, and visualizing the evolution of a research field: A practical application to the Fuzzy Sets Theory field. *J. Inf.* **2011**, *5*, 146–166. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).