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SENSITIVITY AND SPECIFICITY OF rRT-PCR, HISTOPATHOLOGY, AND IMMUNOHISTOCHEMISTRY FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS IN NATURALLY-INFECTED CATTLE AND SHEEP

by

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Submitted in partial fulfilment of the requirements for the degree

of

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DECLARATION

I, **Lieza Odendaal,** hereby declare that the work on which this thesis is based is my own independent work and that neither the whole work nor part of it has been, is being, or shall be submitted for another degree at this or another university, institution for tertiary education or professional examination body.

_____

Lieza Odendaal

_____

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LIST OF ABBREVIATIONS

ABC: Avidin-biotin complex

ARC-OVI: Agricultural Research Council-Onderstepoort Veterinary Institute

BHV-1: Bovine herpesvirus 1

BSA: Bovine serum albumin

CI: Credibility interval

EHV-1: Equine herpesvirus 1

ELISA: Enzyme-linked immunosorbent assay

EMH: Extramedullary haematopoiesis

HE: Haematoxylin and eosin

HIER: Heat-induced epitope retrieval

IBR: Infectious bovine rhinotracheitis

IHC: Immunohistochemistry

NICD: National Institute for Communicable Diseases

Np: Nucleoprotein encoded by the S segment of Rift Valley fever virus

NSs: Non-structural protein encoded by the S segment of Rift Valley fever virus

PBS: Phosphate buffered saline

PBS/BSA: Phosphate buffered saline containing 0.1 % bovine serum albumin

PIER: Protease-induced antigen retrieval

PCR: Polymerase chain reaction

rPCR: Real-time polymerase chain reaction

rtPCR: Reverse transcription-polymerase chain reaction

rRT-PCR: Real-time reverse transcription-polymerase chain reaction

RVF: Rift Valley fever

RVFV: Rift Valley fever virus

WAHID: World animal health information database

WBD: Wesselsbron disease

WBV: Wesselsbron virus

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SUMMARY

SENSITIVITY AND SPECIFICITY OF rRT-PCR, HISTOPATHOLOGY, AND IMMUNOHISTOCHEMISTRY FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS IN NATURALLY-INFECTED CATTLE AND SHEEP

by

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Rift Valley fever (RVF) is a mosquito-borne zoonotic disease caused by a virus of the family Bunyaviridae, genus *Phlebovirus.* It is responsible for extensive outbreaks of disease in livestock in Africa with significant mortality and economic impact. Virus neutralization is considered the gold standard for confirming Rift Valley fever virus (RVFV) infection but the procedure is time consuming and expensive. Real-time reverse transcription-polymerase chain reaction (rRT-PCR), histopathology, and immunohistochemistry (IHC) are the diagnostic methods most often used in South Africa to confirm or exclude a diagnosis of RVF in necropsied animals. Validated estimates of diagnostic accuracy of these tests, in naturally infected livestock, however, have not been published. The objective of this study

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was to estimate the diagnostic sensitivity and specificity of rRT-PCR, histopathology, and IHC using Bayesian latent class methods in the absence of a gold standard. A secondary objective was to estimate stratum-specific values based on species, age, degree of specimen autolysis, and the presence/absence of tissue pigments.

The Sensitivity (Se) and Specificity (Sp) of qRT-PCR were 97.4% (95% credibility interval (CI): 95.2% - 98.8%) and 71.7% (95% CI: 65% - 77.9%) respectively. The extraordinary analytical sensitivity of PCR makes this test very susceptible to false positive reactions, and thus reduced specificity. This is more likely during large-scale epidemics due to cross-contamination of specimens at necropsy facilities or testing laboratories.

The Se and Sp of histopathology were 94.6% (95% CI: 91% - 97.2%) and 92.3% (95% CI: 87.6% - 95.8%) respectively. Single cases of RVF could be confused with acute poisoning with plants, bacterial septicaemias, and viral diseases such as infectious bovine rhinotracheitis and Wesselsbron disease. Most of these conditions, however, can be excluded using histological examination of the liver, special stains, bacterial culture, and toxicological or serological investigations.

The Se and Sp of IHC were 97.6% (95% CI: 93.9% - 99.8%) and 99.4% (95% CI: 96.9% - 100%) respectively. Immunohistochemistry is highly specific because characteristic positive immunolabelling of the cytoplasm of hepatocytes can be correlated with the presence of hepatocellular injury typical for RVFV infection. False negative results are sometimes obtained with IHC because of reader error or loss of the antigenic epitopes due to advanced autolysis. Scant positive immunolabelling might be missed or viral proteins might be absent from sections of liver with advanced hepatocellular damage.

The stratified analysis suggested differences in test accuracy in foetuses and severely autolysed specimens. The Sp of histopathology in foetuses (83.0%) was 9.3% lower than the value obtained for the sample population (92.3%). Lesions in some foetuses are more subtle and the typical eosinophilic intranuclear inclusions are often difficult to detect. In severely autolysed specimens, the Se of IHC decreased by 16.1% and the Sp of rRT-PCR

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by 17.4%. There is no plausible biological explanation for this decrease in the Sp of rRT-PCR since the RNA of RVFV is resistant to degradation in autolysed tissues. Conversely, the antibody used to detect RVFV using IHC detects epitopes raised against nucleoproteins of the virus and it is possible that viral proteins become too widely dispersed and/or degraded in autolysed tissues to detect by light microscopy. It is possible that the marked decrease in Se of histopathology and IHC in severely autolysed specimens caused an apparent decrease in Sp of rRT-PCR, due to the latent class method.

In conclusion, the high estimated Sp (99.4%) of IHC and the low Sp of rRT-PCR (71.3%) suggests that the definitive diagnosis or exclusion of RVF should not rely on a single PCR test and that IHC would be an effective confirmatory test for rRT-PCR positive field cases necropsied during an epidemic. Immunohistochemistry results from severely autolysed specimens, however, should be interpreted with caution and aborted foetuses in areas endemic for RVF should be screened using a variety of tests. The diagnostic Se and Sp of histopathology was much higher than expected confirming the value of routine post mortem examinations and histopathology of liver specimens. The most feasible RVF testing option in areas that do not have suitably equipped PCR laboratories, and where disease is often not detected in livestock until after human cases have been diagnosed, would be routine histopathology screening with IHC confirmation.

Key Words: Rift Valley fever; Rift Valley fever virus; Bayesian; latent-class model; real-time reverse transcription-polymerase chain reaction; immunohistochemistry; histopathology; diagnosis; sensitivity; specificity.

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CHAPTER 1

INTRODUCTION

Rift Valley fever (RVF) is a mosquito-borne zoonotic disease caused by a virus of the family Bunyaviridae, genus *Phlebovirus.* It is responsible for extensive outbreaks in livestock of Africa with significant mortality and economic impact (Pepin *et al.* 2010). The ecology of the disease involves an endemic and an epidemic transmission cycle (Swanepoel & Coetzer 2004). Evidence of low levels of virus transmission has been confirmed in domestic ruminants, wild ruminants, and humans in endemic areas (Bird *et al.* 2009). Sporadically, epidemics of RVF occur over large areas following exceptionally heavy rains that cause substantial increases in vector-competent mosquito populations (Soti *et al.* 2012). In livestock, especially sheep and cattle, epidemics of RVF are characterised by high numbers of abortions (90% to 100% of pregnant ewes in all stages of gestation), sudden death in young animals and severe liver pathology (Bird *et al.* 2009, Swanepoel & Coetzer 2004). Disease susceptibility varies with age and species. Peracute hepatic disease occurs in lambs and calves less than one month old, with estimated mortality proportions as high as 90% to 100% in lambs and 10% to 70% in calves (Bird *et al.* 2009). Mortality in adult ruminants is approximately 10% to 30% in sheep and 5% to 10% in cattle (Bird *et al.* 2009).

The majority of pathological lesions occur in the liver and are similar in all ruminants, but the extent of liver involvement varies with age (Coetzer & Ishak 1982, Coetzer 1977, Coetzer 1982, Daubney, Hudson & Garnham 1931, Easterday *et al.* 1962a, Easterday, Murphy & Bennett 1962b, Findlay 1932). Gross changes in the liver include enlargement with sharply defined pale or dark red foci ranging in size from pinpoint to several millimetres in diameter. The principal histological lesion associated with RVF is random foci of apoptosis (Smith *et al.* 2010, Stalker & Hayes 2007) accompanied by haemorrhage and a mild infiltrate of neutrophils and mononuclear inflammatory cells. Hepatotropic viruses, acute poisoning with a variety of hepatotoxic plants and bacterial septicaemias can present with hepatic lesions

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that could resemble those associated with RVF (Swanepoel & Coetzer 2004). The World Animal Health Organization (OIE) recommends the use of virus isolation, agar gel immunodiffusion, nucleic acid amplification techniques, and immunolabelling to confirm the presence of RVFV (OIE 2012). Specific antibodies to RVFV (Rift Valley fever virus) are demonstrable by virus neutralisation, enzyme-linked immunosorbent assay (ELISA), and haemagglutination inhibition (OIE). Virus neutralization is considered the gold standard for confirming RVFV infection (Pepin *et al.* 2010), but the procedure is time consuming and expensive making it unsuitable for use during an epidemic when the disease is spreading rapidly. Real-time reverse transcription-polymerase chain reaction (rRT-PCR), histopathology, and immunohistochemistry (IHC) are the diagnostic methods most often used in South Africa to confirm or exclude a diagnosis of RVF in necropsied animals. Numerous nucleic acid amplification assays have been developed and evaluated for detection of RVFV, (Pepin *et al.* 2010, Drolet *et al.* 2012, Espach *et al.* 2002) and IHC has been used to detect the distribution of viral antigen in the tissues of lambs and calves (Drolet *et al.* 2012, Rippy *et al.* 1992, Van Der Lugt, Coetzer & Smit 1996). Validated estimates of diagnostic accuracy in naturally infected livestock, however, have not been published. Therefore, the objective of the present study was to estimate the diagnostic sensitivity and specificity of rRT-PCR, histopathology, and IHC using Bayesian latent class methods. A secondary objective was to estimate stratum-specific values based on species, age, degree of specimen autolysis, and the presence/absence of tissue pigments.

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CHAPTER 2

LITERATURE REVIEW

2.1 Etiological agent

Rift Valley fever virus (RVFV) is classified as a single serotype of a bunyavirus in the genus *Phlebovirus*. The complete virion has an icosahedral structure and consists of three RNA genomic segments surrounded by a capsid and a double layered lipid-membrane envelope (Howard 2005). The genome of RVFV consists of two negative-stranded segments (L and M) and one ambisense RNA genomic segment (S) (Howard 2005, Flick & Bouloy 2005).

The large (L) RNA segment encodes the RNA dependent RNA polymerase. The M genomic segment encodes the two major structural glycoproteins, G_N and G_C, as well as NSm (non-structural protein encoded by the M segment) and a 78-kDa protein (Suzich, Kakach & Collett 1990). The small (S) segment has an ambisense strategy and encodes two nucleoproteins namely N and NSs (non-structural protein encoded by the S segment) (Giorgi *et al.* 1991). NSs proteins form filamentous structures in infected hepatocytes that are visible under standard light microscopy as rod-shaped or oval eosinophilic intranuclear inclusions (Mansuroglu *et al.* 2010). The virus capsid is encoded by the viral genome and is formed from identical protein subunits called capsomeres. In the case of RVFV, the capsid is formed from twelve round to hexagonal capsomeres composed of virus glycoproteins named G_N and G_C (Sherman *et al.* 2009). Antibodies used in most ELISAs and IHC tests recognise epitopes of the structural glycoproteins, G_N and G_C or nucleoproteins encoded by the S segment.

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2.2 Epidemiology and ecology

The ecology of RVF disease involves two distinct transmission cycles namely the enzootic transmission cycle in endemic areas and the epizootic transmission cycle in epidemic areas (Swanepoel & Coetzer 2004). The enzootic transmission cycle occurs in inland watershed plateaus or coastal areas during years of normal or below average rainfall. The soil in these areas is relatively non-porous allowing water to stand for weeks or months. In these enzootic areas endemnicity is associated with transovarial transmission of virus in floodwater-breeding Aedes mosquitoes or the overwintering of infected adults (Swanepoel & Coetzer 2004). Low level of virus activity in endemic areas is triggered by rain every year. However, disease is only detected by intensive monitoring. When rainfall occurs, shallow depressions in the landscape, called pans, fill with water and allow floodwater-breeding Aedes mosquitoes to lay their eggs. The eggs are laid in the mud at the edge of the water and require drying before they will hatch prior to the next flooding episode. The life cycle of Aedes mosquitoes can be completed in 10-20 days but the eggs can survive for years in dry mud. Transovarial transmission of virus occurs in a low proportion of infected Aedes mosquitoes and infected eggs are a mechanism for perpetuation of RVFV in enzootic areas. Infected eggs hatch and adult Aedes emerge when the pans fill with water during the rainy season. Infected mosquitoes then transmit the infection to mammals. Evidence of virus transmission has been confirmed in domestic ruminants and equids (e.g. cattle, sheep, goats, camels, and horses), wild ruminants (e.g. African buffaloes, zebras, black rhino, elephants, warthogs, impala, and waterbuck), and humans (Bird *et al.* 2009). Normal rainfall patterns greatly limit the period available for vector activity and disease transmission. This limits the extent of outbreaks in enzootic areas (Swanepoel & Coetzer 2004). Epidemics of RVF are caused by exceptionally heavy rains and occur in the same areas where endemic virus activity occurs. During years of abnormally high rainfall rivers flood their banks, pans fill with larger amounts of water, and large pools of stagnant water are available. During these times extensive swarms of mosquitoes emerge, and with sufficient numbers of

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susceptible unvaccinated livestock such as cattle and sheep in the same area as RVFV-infected mosquitoes, epidemics occur. Culex mosquitoes and biting flies are also involved in RVFV transmission during epidemics (Swanepoel & Coetzer 2004). These vectors acquire virus by taking blood meals from viraemic ruminants and function as virus amplifiers. The virus is subsequently disseminated over an ever-widening area, thereby worsening the epidemic. Culex mosquitoes do not serve as a reservoir for RVFV since they are unable to transmit the virus transovarially and they require the availability of stagnant water such as dams or marshes over weeks or months to complete their life cycle (Pepin *et al.* 2010).

Rift Valley fever occurs predominantly in Sub-Saharan Africa. Large outbreaks of RVF occurred in South Africa from 1950 to 1951 and again from 1974 to 1976 (Pienaar & Thompson 2013). Fatal human disease was recognized for the first time during the outbreak in the 1970s (Swanepoel, Manning & Watt 1979). The largest RVF epidemic affected Egypt along the Nile River from 1977 to 1979, affecting approximately 200,000 people including 594 deaths, followed by recurrent epidemics in 1993 and 1997 (Abd El-Rahim, Abd El-Hakim & Hussein 1999, Abu-Elyazeed *et al.* 1996, Arthur *et al.* 1993, Meegan 1979, Meegan, Hoogstraal & Moussa 1979). Senegal-Mauritania, in West Africa, had severe outbreaks in 1987 where an estimated 89,000 people were affected including 220 deaths (Digoutte & Peters 1989). An outbreak in East Africa occurred from 1997 to 1998 in North-eastern Kenya, affecting an estimated 27,500 individuals with 170 deaths (LaBeaud *et al.* 2008, Woods *et al.* 2002). Epidemics of RVF occurred in Madagascar in 1990 and 1991 and 2008 and 2009, causing only a few deaths in 1990 and 1991 but 520 cases and 20 deaths in 2008 (Andriamandimby *et al.* 2010, Morvan *et al.* 1991, Morvan *et al.* 1992). Rift Valley fever spread to the Arabian peninsula in 2000 (Saudi Arabia and Yemen), where an estimated 2000 humans were hospitalized or severely debilitated with RVF-related symptoms, and causing at least 245 deaths (Al-Hazmi *et al.* 2003, Madani *et al.* 2003). It then re-emerged in Egypt in 2003 (Balkhy & Memish 2003). In recent years RVF has circulated in East Africa since 2007 causing serious epidemics in Kenya, Tanzania, Somalia, and Sudan (Bird *et al.*

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2008, Breiman *et al.* 2008, Hassan *et al.* 2011, Jost *et al.* 2010). Rift Valley fever activity was also reported in Mayotte from 2007 to 2008 (Cêtre-Sossah *et al.* 2012). The disease subsequently emerged South Africa (Pienaar & Thompson 2013).

In South Africa, RVF occurred in small numbers of cattle, sheep, goats, and African buffalo in Mpumalanga, North West, Gauteng, and Limpopo provinces from January 2008 to June 2008 (Pienaar & Thompson 2013). Eighteen laboratory-confirmed cases of RVF in humans were reported during this period (Pienaar & Thompson 2013). Small isolated outbreaks of RVF were again reported in KwaZulu-Natal and Mpumalanga during February to June 2009 (Pienaar & Thompson 2013). Cattle and sheep were affected but no confirmed human cases were linked to this outbreak. In October and November 2009 a small outbreak occurred in cattle, sheep, and goats in the Northern Cape adjacent to the Orange River (Pienaar & Thompson 2013). In this instance two human cases were confirmed. An extensive outbreak of RVF affecting livestock and humans occurred in 2010 (Pienaar & Thompson 2013). Cases were reported on farms predominantly in the Free State Province but also occurred in the Eastern Cape, Northern Cape, Gauteng, Mpumalanga, North West, and Western Cape Provinces. A total of 242 laboratory-confirmed cases of RVF in humans with 26 deaths were identified in South Africa during 2010 (Blumberg *et al.* 2011). In 2011 a smaller outbreak occurred in sheep, goats, cattle and wild animals in the Eastern Cape, Northern Cape and Western Cape Provinces (Pienaar & Thompson 2013).

2.3 Pathology of Rift Valley fever in ruminants

Macroscopic and microscopic lesions pertaining to RVF have been quite extensively described (Coetzer & Ishak 1982, Coetzer 1977, Coetzer 1982, Daubney *et al.* 1931, Easterday *et al.* 1962a, Easterday *et al.* 1962b, Findlay 1932, Findlay 1933).

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The most consistent macroscopic findings are hepatic lesions, widespread subcutaneous and serosal haemorrhages, and severe pulmonary oedema and congestion (Swanepoel & Coetzer 2004). This is often accompanied by enlargement and congestion of peripheral and visceral lymph nodes and mild to moderate hydrothorax, hydropericardium, and serosanguinous ascites (Swanepoel & Coetzer 2004). Icterus can be evident, especially in adult sheep (Swanepoel & Coetzer 2004).

The macroscopic hepatic lesions are similar in all ruminants, but the extent of liver involvement varies with age (Swanepoel & Coetzer 2004). Lesions are most severe in aborted foetuses and new-born lambs in which the liver is yellow, swollen, and friable (Swanepoel & Coetzer 2004). In older animals the liver tends to be darker with discrete greyish-white or dark-red foci ranging in size from pinpoint to several millimetres in diameter (Swanepoel & Coetzer 2004). Lesions are extensive in newborn lambs and adult sheep where obvious discrete necrotic foci are sharply delineated from the adjacent parenchyma giving the liver a mottled appearance (Swanepoel & Coetzer 2004). Usually, only scattered hepatic lesions are seen in the livers of adult cattle while more numerous lesions are discernable in calves (Swanepoel & Coetzer 2004).

Microscopic pathology occurs predominantly in the liver but the extent of hepatocellular injury varies with age (Swanepoel & Coetzer 2004). Adult animals more often present with random multifocal to coalescing areas of hepatocellular death while lambs and foetuses tend to present with massive hepatocellular death (Swanepoel & Coetzer 2004). The lesions are essentially the same in cattle, sheep, and goats (Coetzer & Ishak 1982, Coetzer 1977, Coetzer 1982, Easterday *et al.* 1962a, Easterday *et al.* 1962b, Findlay 1932, Findlay 1933). Experimental infections in lambs caused only isolated degeneration or death of hepatocytes 18 hours post infection (Coetzer & Ishak 1982, Easterday *et al.* 1962b). Randomly distributed primary foci of hepatocellular injury follow 18 to 24 hours post infection (Coetzer & Ishak 1982, Easterday *et al.* 1962b). These foci consist of inflammatory cells and prominent hepatocyte death due to cytolysis. Within hours the primary foci enlarge and

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consist of inflammatory cells and basophilic karyorrhectic remnants of nuclei against a background of homogenous eosinophilic debris (Easterday *et al.* 1962b). These primary foci are considered pathognomonic for RVFV infection and feature more prominently in foetuses and neonates (Swanepoel & Coetzer 2004). At 24 hours most of the hepatocytes in the lobules (in foetuses and neonates) undergo apoptosis preserving only the portal triads. Apoptosis of RVFV infected hepatocytes are recognised, and Findlay (1932) gives a particularly vivid description of the cytopathology caused by RVFV, with hallmark features of apoptosis (Smith *et al.* 2010, Stalker & Hayes 2007, Findlay 1933). These include dissociation of cells, shrinkage, and rounding of hepatocytes with hypereosinophilic cytoplasm, and karyorrhexis or karyolysis. Hepatocyte nuclei undergo degeneration and chromatin margination (Findlay 1932). A portion or portions of the cytoplasm become intensely eosinophilic, rounded hyaline globular masses (autophagosomes) that eventually separate off from the portion of the cytoplasm which contains the degenerating nucleus, forming an acidophilic (apoptotic) body (Findlay 1932). The whole of the cytoplasm may also be involved so that the cell becomes a hypereosinophilic, rounded, or oval hyaline (apoptotic) body (Findlay 1932). Some remains of the nucleus may still be found within the apoptotic body but, more commonly the nucleus has completely disappeared (Findlay 1933).

Small groups of surviving hepatocytes, often showing fatty, or hydropic degeneration may be present in the vicinity of the portal triads or within the limiting plate (Coetzer & Ishak 1982). This is accompanied by disseminated haemorrhage, frequently more pronounced in the centrilobular sinusoids and around the larger intercalating and collecting veins (Coetzer 1977). Scattered neutrophils accompanied by smaller numbers of Kupffer cells are frequently present between the apoptotic cells (Coetzer 1977). Hepatocyte nuclei are usually still identifiable in degenerated hepatocytes. These are vesicular with marginated chromatin and may contain filamentous, rod-shaped, oval, or round eosinophilic intranuclear inclusions in approximately 50% of the livers of neonatal lambs and 20% of the livers of adult cattle (Coetzer 1977, Coetzer 1982). Fibrin deposition in the sinusoids is also a common

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finding (Coetzer & Ishak 1982, Coetzer, Ishak & Calvert 1982). Less frequently seen microscopic lesions include vasculitis and thrombosis, mineralisation of scattered or small groups of injured hepatocytes, mild infiltration of the portal tracts with mononuclear cells some of which show cytolysis, mild oedema in the portal tracts, and mild limiting plate proliferative activity (Coetzer 1977).

Microscopic lesions are also present in other organs including the spleen, lymph nodes, kidneys, lungs, adrenal glands, and digestive tract. Mild to marked lymphocytolysis in lymphoid tissues and congestion and oedema in the lungs are found in most cases (Swanepoel & Coetzer 2004). Less frequently seen is cloudy swelling and hydropic degeneration of the epithelial cells of the proximal tubules of the kidneys, necrosis of some of the cellular elements in glomeruli (approximately 10% of lambs), haemorrhages in the submucosa of the gastrointestinal tract, and multifocal necrosis and haemorrhage in the adrenal cortex (Swanepoel & Coetzer 2004). Deposits of fibrin may be seen in the hepatic sinusoids, glomeruli, lymphoid tissues and alveolar walls (Swanepoel & Coetzer 2004).

2.4 Diagnostic techniques

Microscopic examination of liver specimens is a useful technique for the preliminary diagnosis of RVF. The diagnosis, however, should always be confirmed by demonstrating the presence of specific antigen of RVFV genome (OIE 2012). Numerous diagnostic techniques to detect the presence of RVFV antigen have been described and some of the traditional methods such as agar gel immunodiffusion, older formats of enzyme-linked immunosorbent assays, and immunofluorescence carried out on impression smears of liver, spleen and brain have been replaced in recent years by more rapid and accurate tests. The most commonly employed techniques include rRT-PCR and IHC. These tests are reviewed in more detail.

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2.4.1 Polymerase chain reaction

RNA of RVFV may be rapidly detected by rRT-PCR (Pepin *et al.* 2010, Espach *et al.* 2002, Bird *et al.* 2007, Drosten *et al.* 2002, Njenga *et al.* 2009, Roux *et al.* 2009) PCR has been extensively adapted for numerous molecular biology applications and the following is a short summary of the basic PCR assay and some variations on the technique that have been developed to detect and quantify RNA.

RNA, converted into complimentary DNA by the reverse transcription reaction can be detected using real-time polymerase chain reaction (Sambrook & Green 2012). The standard method is performed in a thermal cycler equipped with a system to detect and quantify RNA transcripts (Sambrook & Green 2012). Polymerase chain reaction (PCR) entails the use of two primers, each about 20 nucleotides in length (Burtis & Ashwood 2001). The one oligonucleotide is complementary to a short defined sequence in one strand (the sense strand) of the DNA to be amplified, and the other is complementary to a sequence in the other DNA strand (the anti-sense strand). The basic technique involves the addition of large quantities of primers specific for the genomic segment of interest into a solution that contains a heat-stable DNA polymerase (such as Taq polymerase), deoxynucleoside triphosphates, divalent cations (usually Mg^{2+}), monovalent cations (KCL), and a suitable buffer (Tris-Cl, adjusted to a pH between 8.3 and 8.8) in which the DNA containing the target sequences is heated (denatured) to separate the strands (Sambrook & Green 2012). The denaturation step yields two single-stranded DNA molecules (Berg, Tymoczko & Stryer 2005). This is followed by the annealing and elongation steps (Berg *et al.* 2005). As the solution is cooled, the primers form base pairs (anneal) with the DNA. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The annealed DNA strands then serve as primers for the synthesis of more DNA strands by the heat-stable DNA polymerase (elongation). The process of heating, cooling, and new DNA synthesis is repeated many times (amplified) until a large

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number of copies of the DNA are obtained. After 30 to 40 rounds of amplification in the thermal cycler, the reaction product is analysed to check whether the PCR generated the anticipated DNA fragment (also sometimes referred to as the amplimer or amplicon) (Berg *et al.* 2005)

The standard method to detect, analyze, and quantify the amplicon is real-time PCR (rPCR) (Sambrook & Green 2012). This method was developed to enable both detection and quantification of specific sequences in a specimen. The key feature of rPCR is that the amplified DNA is detected and quantified as the reaction progresses in real time. A common method for detection and quantification of products in rPCR is non-specific fluorescent markers such as SYBR green that intercalate with any double-stranded DNA (Sambrook & Green 2012). However, SYBR green will bind to all double-stranded DNA PCR products, including nonspecific PCR products such as primer-dimers which can potentially interfere with, or prevent, accurate quantification of the intended target sequence. An alternative method to detect PCR product is with a sequence-specific DNA probe (TaqMan) consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary DNA target (VanGuilder, Vrana & Freeman 2008). The probe consists of a sequence of oligonucleotides labelled with a fluorescent reporter and a quencher attached to each end of the probe which is maintained in close proximity until hybridization of the probe with its complementary DNA target occurs. Taq polymerase contains a polymerase domain for synthesizing a new DNA strand and a 5' nuclease domain that is able to cleave RNA primers or probes (Lyamichev *et al.* 1999). The probe is hydrolyzed by the 5′ nuclease activity of the Taq polymerase following annealing of the primers and the probes to their respective target sequences, causing separation of the fluorescent reporter and the quencher which causes an increase in fluorescence intensity because the reporter is no longer quenched (Livak *et al.* 1995, Livak 1999). A proportional increase in fluorescence intensity at each PCR cycle, detected after excitation with a laser,

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indicates that the probe-specific PCR product has been generated. Alternatively, the reporter remains quenched if PCR product is not present and the probe cannot hybridize to its target sequence. Fluorescent markers emit light in proportion to the amount of PCR product generated during the amplification reaction and measurements of fluorescence intensity, made in real time, are analysed using computer software to quantify the amplified product (Sambrook & Green 2012).

2.4.2 Immunohistochemistry

Immunohistochemistry (IHC) is an inexpensive and rapid procedure that identifies antigens in routinely processed paraffin wax-embedded tissues with an antibody and an appropriate labelling system (Ramos-Vara *et al.* 2008). IHC is especially advantageous as a diagnostic tool for infectious diseases since it allows co-localization of an antigen with a lesion thereby increasing the specificity (Ramos-Vara *et al.* 2008). For some diseases (e.g. prion diseases), IHC is considered the gold standard (Ramos-Vara *et al.* 2008).

2.4.2.1 Detection antibodies

Antibodies used to detect specific antigens can be polyclonal or monoclonal, and are produced by immunizing animals (mice, rabbits, goats, horse, chickens, etc) with purified antigen (Ramos-Vara 2005). Monoclonal antibodies are derived from B-lymphocytes hybridized with myeloma cells, which produce immunoglobulins that bind to a single epitope on one antigen, whereas polyclonal antibodies are isolated from whole serum and recognize multiple epitopes on target proteins (Ramos-Vara 2005). Polyclonal antibodies therefore have higher sensitivity due to multiplicity of epitope recognition, but sometimes have lower specificity when compared to monoclonal antibodies due to the greater likelihood of cross reactivity with similar epitopes on other proteins (Ramos-Vara 2005).

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2.4.2.2 Immunohistochemistry protocols

Standard IHC protocols include fixation, deparaffinising of paraffin embedded tissues, rehydrating of formalin-fixed paraffin embedded tissue sections followed by antigen retrieval, immunohistochemical labelling, antigen-antibody reaction visualization, counterstaining, dehydration, stabilizing with a mounting medium, coversliping and viewing the labelling with a standard light microscope (Ramos-Vara 2005).

To begin with, samples are prepared by fixation. Fixation is critical to maintain the antigenicity of target epitopes by preserving the general architecture of tissues and the structure of cellular organelles (Ramos-Vara *et al.* 2008). The most commonly used fixative is formaldehyde which is capable of forming cross-links with amino acid groups (Ramos-Vara *et al.* 2008). This changes the conformation of antigenic glycoproteins or polypeptides necessitating the incorporation of an antigen retrieval step in the protocol (Ramos-Vara *et al.* 2008). Two methods of antigen retrieval for infectious diseases are generally used at the Faculty of Veterinary Science, University of Pretoria namely, heat-induced epitope retrieval (HIER) or protease-induced antigen retrieval (PIER). The method used depends on the specific target antigens. For instance, antigenic epitopes for Wesselsbron virus (WBV) and RVFV are best detected using HIER whereas PIER yields better results for bovine herpesvirus 1 (BHV-1) or equine herpesvirus 1 (EHV-1).

Samples for IHC are usually also obtained from tissues that were embedded in paraffin. Generally, typical lesions for infectious diseases are detected upon histological examination of tissues. Thereafter, 4 micrometer-thick tissue sections cut from formalin-fixed paraffin embedded tissues must be deparaffinised with xylene and rehydrated through graded alcohol baths (100%, 96%, and 70% ethanol) to distilled water. In addition, tissues such as kidney, liver, or vascular areas with red blood cells, contain endogenous peroxidase activity that causes non-specific background staining (Miller 2002). This is suppressed using 3% hydrogen peroxide in methanol, following which antigen is retrieved in a suitable buffer. The buffer in which antigen retrieval and further tissue processing is performed determines

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whether antibodies successfully bind to antigens (Ramos-Vara *et al.* 2008). The most popular buffers are tri-sodium citrate (pH6) and TrisEDTA (pH9). Choice of retrieval buffer depends on the properties of the antigenic epitope and is best determined by experimentation. Following antigen retrieval slides are rinsed in distilled water and then incubated in phosphate buffered saline (PBS) (pH 7.6) that contains a blocking agent. Blocking of epitopes, to which antibodies may otherwise bind, is necessary to prevent non-specific immunolabelling. Any protein that does not have binding affinity for the target or probe components in the assay can be used for blocking, and common blocking agents include commercially available normal serum (a serum from the species that the secondary antibody was generated in), bovine serum albumin, gelatin or non-fat dry milk (Snider 2013). Following blocking, the serum is drained and replaced by the primary antibody buffered in phosphate buffered saline/bovine serum albumin (PBS/BSA).

All incubations should be carried out in a humidified chamber to avoid desiccation of the tissue. Desiccation at any stage will lead to non-specific binding and ultimately high background staining (Ramos-Vara *et al.* 2008). Dilutions of the primary antibody and ideal incubation times are determined by testing a range of possibilities.

To visualize the reaction the primary antibody is labelled with a secondary or link antibody (Ramos-Vara *et al.* 2008). Commercially available rabbit-anti-mouse or goat-anti-rabbit secondary antibodies are often used. The choice of secondary antibody depends on the animal species in which the primary antibody was raised. Rabbit-anti-mouse antibody is used in the case of RVFV antibody since the latter was produced in mice. The most commonly used detection systems are avidin-biotin peroxidase and polymer based systems. Biotin is attached to the secondary antibody (biotinylated) that binds a complex of avidin-peroxidase and produces a coloured reaction. Suppression of endogenous peroxidase activity, prior to immunohistochemical labelling, is essential with this detection method. The polymer based detection system does not require this step since it does not involve peroxidase. Instead, the primary antibody is labelled with a polymer that contains

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numerous secondary antibodies and enzyme molecules. The choice of system depends on factors such as the type of antigen to be detected or cost. The avidin-biotin peroxidase system is sufficient for RVFV detection whereas the polymer based system is more effective for WBV.

All sections are immersed in a NovaRED substrate for peroxidase following labelling with the secondary or link antibody. This produces a red reaction product for the purpose of contrasting brownish tissue pigments (especially bile, lipofuchsin or haemosiderin in liver sections). The positive control section is also treated with the NovaRED substrate at the same time and examined for signs of positive labelling using a light microscope. All sections are immediately rinsed with distilled water to stop the reaction as soon as there is clear evidence of a positive reaction in the control section. A counterstain is often applied and the most commonly used is Mayer's haematoxylin. This stains the tissues blue and provides a contrast to the red stained antigen-antibody complexes allowing for co-localisation of positive labelling with histopathologic lesions.

The distribution of viral antigen in tissues of new-born lambs infected with RVFV was studied by van der Lugt *et al.* (1996) (Van Der Lugt *et al.* 1996). This study used an avidin-biotin complex (ABC) immunoperoxidase technique. Virus antigen was primarily detected in the liver. Diffuse granular labelling was observed in the cytoplasm of affected hepatocytes but not in the nuclei. This is to be expected since RVFV replicates in the cytoplasm, while the viral proteins transit through the endoplasmic reticulum and Golgi apparatus, and mature virions bud from the Golgi apparatus into vesicles which are transported to the cell surface (Gerrard & Nichol 2002). Small groups of injured hepatocytes labelled positive for RVFV in lambs euthanized early in the course of the disease while viral antigen was widely dispersed in injured hepatocytes throughout the liver lobules in lambs euthanized later on or in lambs killed in extremis. Van der Lugt *et al.* (1996) also found that the labelling of foci of necrosis becomes less intense during the late or terminal stages of the disease possibly due to the release of virus from completely destroyed cells (Van Der Lugt *et al.* 1996).

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2.4.3 Diagnostic challenges

A major diagnostic challenge is that RVF in domestic ruminants is difficult to distinguish from diseases caused by other agents. These agents cause lesions that superficially resemble those caused by RVFV and include poisoning with plants such as *Senecio spp*., *Crotalaria spp*., *Lasiospermum bipinnatum*, *Cestrum spp*., *Pteronia pallens* and *Hertia pallens*, and an alga, *Microcystis aeruginosa*, as well as bacterial septicaemias such as pasteurellosis, salmonellosis and anthrax (Swanepoel & Coetzer 2004). In addition, outbreaks of abortions in livestock can be ascribed to RVFV infection. However, other abortifacient agents include *Brucella spp*., *Leptospira spp*, *Chlamydia spp*., *Campylobacter spp.* and *Coxiella burnetti* infection (Swanepoel & Coetzer 2004).

A further diagnostic challenge is that the accuracy of the diagnostic tests that are most commonly employed can be compromised by various factors. Conceivably, false negative results are sometimes obtained from these tests due to loss of the antigenic epitopes caused by advanced autolysis, necrosis, prolonged fixation of specimens in formalin, fixative with a pH of 6 or less and the length of the tissue processing cycle (Chung *et al.* 2008). The process of formalin fixation causes protein–protein cross-links, owing to reactivity of formaldehyde with radicals or residues of the amino acid lysine, arginine, tyrosine, aspartate, histidine, and serine (Ramos-Vara 2005). There are also concerns about PCR in a diagnostic setting. The high analytical sensitivity of PCR makes this test susceptible to false positive results that may occur if the sample is contaminated either during sample collection or during analysis (Burtis & Ashwood 2001). Post-mortem examination facilities may be inundated with cases to examine during a RVF outbreak and many carcasses of animals that originate from different farms may be examined simultaneously in the same room. Under these circumstances, aerosol contamination of specimens is likely. Similarly, the laboratory must also test large volumes of samples and aerosol droplets of the product of the amplification reaction (the amplicon) can contaminate samples, reagents, or glassware. False-negative results on PCR assays also occur when primer sequences are not entirely

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complementary to the target sequence or when primers fail to bind and amplification fails despite the presence of the target sequence (Saah & Hoover 1997). This phenomenon can occur with RVFV due to genetic variation in the genome of the virus with 40 ecologically and biologically diverse virus isolates identified (Bird *et al.* 2007).

These threats and diagnostic challenges prompt an increasing need for accurate and rapid techniques to diagnose the disease and enable effective surveillance for RVF in high risk areas.

2.5 Principles of diagnostic test validation utilizing Bayesian methods

In the absence of a gold standard or prior knowledge concerning the true RVF infection status of the population, sensitivity (Se) and specificity (Sp) of rRT-PCR, histopathology, and IHC cannot be estimated directly (Joseph, Gyorkos & Coupal 1995). Instead, estimation of the diagnostic accuracy of the tests must be estimated in a latent class analysis, and using a Bayesian framework is a common approach (Branscum, Gardner & Johnson 2005, Enøe, Georgiadis & Johnson 2000, Joseph *et al.* 1995, Scott *et al.* 2007, Toft, Jørgensen & Højsgaard 2005). The term “latent” is used and this class of models is termed “latent class” because the existing tests are presumable not sufficiently accurate to qualify as gold standards and therefore the true disease status of individual animals is not observable (i.e. latent) (Walter & Irwig 1988).

Bayesian methods are based on Bayes' theorem that can be written as follows:

$$P(\theta \mid \text{data}) = \frac{P(\text{data} \mid \theta) * P(\theta)}{P(\text{data})} \quad \text{where: } P(\text{data} \mid \theta) = \text{Likelihood function, } L(\theta)$$

In the above formula we allow θ to denote a currently unknown parameter such as diagnostic sensitivity, diagnostic specificity, or disease prevalence. Also, the usual term for p(θ) is the prior, for P(data | θ) the likelihood, and for P(θ | data) the posterior (Spiegelhalter *et al.* 2000). In general, the Bayesian approach to inference about the unknown parameter

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(θ), involves the combination of prior information about the parameter of interest (through the prior probability p(θ)) based on evidence not derived from the study under analysis with information concerning the same parameter available from the data collected in the study (through the likelihood P(data|θ)) to obtain an updated or posterior distribution of the parameter (P(data|θ)) (Branscum *et al.* 2005, Spiegelhalter *et al.* 2000). Stated simply, Bayes's theorem states that the posterior distribution is proportional to the product of the prior multiplied by the likelihood. The mean, median, or mode of this posterior distribution can be used as the point estimate of θ (Branscum *et al.* 2005).

A credibility interval (or probability interval), analogous to a confidence interval, is obtained by finding the values (lower, upper) where the area under the graph of P(θ|data) between these points is 0.95 (or any other selected value) (Branscum *et al.* 2005). Markov Chain Monte Carlo (MCMC) methods have been developed to solve these types of problems. MCMC samples are obtained iteratively and with a large sample, medians and percentiles serve as point estimates and probability intervals, respectively.

Lack of a gold standard creates a situation where sensitivity and specificity of diagnostic tests cannot be estimated directly (Joseph *et al.* 1995). Available information includes the number of animals with each of the possible positive (+) and negative (-) test result patterns. The total count for each test pattern is the sum of the unobserved (latent) number of infected and uninfected animals. The probability of belonging to a category is calculated by the addition of the components corresponding to infected and uninfected animals. For each animal population, the observed table of total counts follows a multinomial distribution with unknown probabilities. Each cell probability is dependent on the infection prevalence and diagnostic test parameters. The multinomial probability for each cell is calculated as a weighted average with contributions corresponding to infected animals (weighted by prevalence) and uninfected animals (weighted by 1 - prevalence). These models can be extended to incorporate results from more than 2 tests, multiple populations, and statistical comparisons of diagnostic accuracy (Branscum *et al.* 2005). Beta probability distributions

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are often used to incorporate prior scientific information, P(θ), and available software (WinBUGS version 1.4, MRC Biostatistics Unit) can estimate posterior probability distributions. Results of this type of analyses include distributions for the prevalence of infection and diagnostic test sensitivity and specificity.

2.6 Estimating repeatability of histopathology and immunohistochemistry

The consensus estimate referred to as Cohen's kappa coefficient (κ) can be used to quantify the Inter-rater agreement (the degree of agreement between the two independent observers of the histopathology and the IHC) (Carletta 1996). This statistic corrects the percentage of agreement estimate by taking into consideration the amount of agreement that would be expected by chance. Therefore, the formula for the κ statistic can be written as follows:

$$\kappa = \frac{P_{agreement} - P_{chance}}{1 - P_{chance}}$$

The κ ratio expresses the corrected proportion of agreement between observers as a ratio of the total proportion of agreements corrected for chance responses (P_{chance}) and is a bounded statistic between -1 and +1. A positive value indicates that proportion of agreement is greater than chance, and a negative value indicates that the proportion of agreement is less than chance. A value of +1 indicates perfect agreement between observers. A κ value of 0 indicates that the observer agreement is indistinguishable from chance.

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CHAPTER 3

MATERIALS AND METHODS

3.1 Outbreak description

An extensive outbreak of Rift Valley fever (RVF) occurred in South Africa during 2010. The first report was made at the end of January and documented the sudden death of 230 young lambs in the Bultfontein and Brandfort areas of the Free State Province (World animal health information database [WAHID]: 2010, South Africa, Immediate Notification) (Pienaar & Thompson 2013, WAHID Interface 2010a). Additional cases (n=80) were reported in the beginning of February and a diagnosis of RVF was confirmed on 18 February 2010 by rRT-PCR and IHC (WAHID: Report date 19/02/2010) (WAHID Interface 2010a). The epidemic rapidly spread throughout the Free State Province and subsequently extended into the Eastern Cape, Northern Cape, Western Cape, and North-West Provinces (Pienaar & Thompson 2013). Isolated outbreaks were also reported in Gauteng, Limpopo and Mpumalanga Provinces with none reported in Kwazulu-Natal. The outbreak affected mainly sheep but also cattle, goats, African buffaloes, camelids, and other wild animals (Pienaar & Thompson 2013). A total of 14342 animal cases and 8877 animal deaths were reported (Pienaar & Thompson 2013). The last animal cases were reported in the Northern Cape Province at the end of August (WAHID: Report date 29/11/2010) (WAHID Interface 2010b). Most farmers reported high abortion rates, dystocia, or acute collapse and death of animals without noticing other clinical signs. In some instances farmers reported the death of up to 100% of newborn lambs. Clinical signs that were occasionally noted include anorexia, dysgalactia, nasal and lachrymal discharges, salivation, colic, jaundice, or haemorrhagic enteritis.

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3.2 Samples size justification

The necessary sample size was calculated for an expected sensitivity and specificity of 0.95, and the desire to estimate this value ±0.05 at the 95% credibility level. A conservative frequentist approach (rather than Bayesian) was employed using exact binomial methods as previously described (Fosgate 2009). The calculated sample size was 97 cases each for RVF infected and uninfected livestock. The true prevalence was unknown and it was expected that there might be a high prevalence in the sample population (~75%) so the calculated sample size was multiplied by 4 in an effort to obtain enough samples from RVF uninfected livestock. All samples from cattle, neonates, and foetuses from all Provinces were included in the study with the balance being adult sheep from the Free State Province to obtain the required sample size. The sample size was increased by approximately 10% to allow for possible sample loss and incomplete diagnostic information.

3.3 Rift Valley fever outbreak specimen selection

This was a retrospective study of cases submitted to the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI), at the behest of the South African Department of Agriculture Fisheries and Forestry (DAFF). All specimens originated from the carcasses of naturally infected animals and foetuses that were necropsied during the 2010 RVF outbreak. Fresh and 10% formalin-fixed specimens were submitted to the ARC-OVI and a private veterinary diagnostic laboratory (IDEXX laboratories, Pretoria) respectively, for the diagnosis of RVF. The rRT-PCR results were made available by the ARC-OVI and these could be correlated with histopathology reports of the private laboratory. Archival paraffin-embedded tissue samples were obtained from the private laboratory, and selected on the basis of a positive or a negative diagnosis of RVF infection by rRT-PCR. Four hundred and twenty individual cases from cattle, sheep, and goats were selected for inclusion in the study. This included 127 cattle, 273 sheep, and 20 goats classified as adult, neonate, or foetus (Appendix 1).

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3.4 Control specimen selection

An additional 40 blinded control cases were obtained from the archives of the Faculty of Veterinary Science, University of Pretoria, for randomization among the 2010 RVF outbreak cases (Appendix 2). These included 8 rRT-PCR and IHC positive cases of RVF that occurred in 2009 and 32 additional cases due to aetiologies that cause liver pathology resembling RVF. In the case of IHC, relevant or irrelevant antibodies were applied to sections to create blinded controls for positive and negative reactions.

Non-RVF controls included 7 cases where hepatotoxic plants caused acute death in adult cattle or sheep, 10 cases of equine herpesvirus 1 infection (EHV-1) in equine foetuses, 4 cases of infectious bovine rhinotracheitis (IBR) in bovine foetuses, 10 cases of Wesselsbron disease (WBD) in neonatal and adult sheep and 1 case of salmonellosis in an adult bovine. Cases of EHV-1 infection were added due to the limited availability of control cases in ruminant foetuses.

The EHV-1, IBR and WBD cases were verified by IHC and characteristic histological lesions. The cases where the aetiology was acute poisoning by a hepatotoxic plant were selected on the basis of positive identification of the plant and characteristic histological lesions. Two cases of poisoning by *Lasiospermum bipinnatum,* one of *Lantana camara,* two of *Xanthuim strumarium,* one of a *Cestrum* spp*,* and one of *Senecio* spp. were included. The case of salmonellosis had characteristic histological lesions verified by bacterial culture.

The purpose of the additional 40 cases was to circumvent the practise of a pathologist to automatically associate certain lesion patterns in organs with the expected findings for a specific disease. In short, the selection of a range of hepatic lesions that are caused by agents other than RVFV and the application of relevant or irrelevant antibodies to these sections prompted the pathologists reading the sections to examine them methodically and not predict the presence or absence of immunolabelling, whether consciously or

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unconsciously, based on the presence or absence of lesions characteristic for RVF in sections labelled with RVFV antibody. The haematoxylin and eosin (HE) stained sections of these 40 cases were used to elicit prior probability distributions for the sensitivity and specificity of histopathology.

3.5 Diagnostic testing

3.5.1 Real-time reverse-transcriptase polymerase chain reaction

Nucleic acid extractions and rRT-PCR were performed by an immunized individual (Dr Marco Romito), under biosecure conditions, at the Biotechnology PCR Laboratory of the ARC-OVI. The assay was optimized for use with 200ul blood or tissue lysate.

RNA was extracted from approximately 0.1 cm^3 liver macerated in tissue lysis buffer using an automated system (MagNA Pure LC Total Nucleic Acid High Performance Kit, catalogue no: 05 323 738 001, used with a MagNA Pure LC system, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany) according to the manufacturer's instructions. As previously described, (Drosten *et al.* 2002) novel sense and antisense primer pairs and a 5' hydrolysis probe, targeting conserved sequences on the G2 gene from the M segment of the viral genome were used.

Reactions were carried out using a commercial kit (LC 480 One-step RNA Master (Hydrolysis Probes) Kit, catalogue no: 04 991 885 001, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany) which incorporates the polymerase enzyme that has both reverse-transcriptase and DNA-polymerase activity. All reactions were carried out on 96-well plates in a real-time thermo cycler (LightCycler® 480 Real-Time PCR System, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany) and each plate included RVFV positive controls (Smithburne and/or Clone 13 vaccine strain: Onderstepoort Biological Products, Pretoria, South Africa), an unrelated RNA control, and a non-template control reaction. Each 10 µl reaction consisted of 0.6 µl $Mn(OAc)_2$, 3.7 µl 2x Hydrolysis Mix (LC 480 One-step RNA Master (Hydrolysis Probes) Kit, catalogue no: 04 991 885 001, Roche

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Diagnostics GmbH, Sandhofer, Mannheim, Germany), 0.5 µl Enhancer (LC 480 One-step RNA Master (Hydrolysis Probes) Kit, catalogue no: 04 991 885 001, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany), 5 pmol of each primer, 1 pmol 5' FAM and 3' BHQ-3 labelled probe, 2 µl nucleic acid extract and water q.s. The cycling profile PCR was run on an automated system (LightCycler® 480 Real-Time PCR System, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany) and involved reverse transcription at 63°C for 3 min, initial denaturation at 95°C for 30 s, and 45 cycles of 95°C for 10 s and 60°C for 30 s. Fluorescence was read at the combined annealing-extension step and results analysed using the supplied system software (LightCycler® 480 Real-Time PCR System, Roche Diagnostics GmbH, Sandhofer, Mannheim, Germany).

Cases were dichotomized as either positive or negative for RVF. Cases where the test result was inconclusive (cycle count>35.00 or an atypical amplification curve i.e. not logistic), but still regarded as suspicious for RVF, were classified as positive.

3.5.2 Histopathology

3.5.2.1 Processing of tissue samples

All tissue samples submitted for microscopic examination were processed at the histotechnology laboratory of the Faculty of Veterinary Science, University of Pretoria. Following dehydration, clearing and infiltration with paraffin wax, formalin fixed tissues were placed into moulds along with liquid wax and hardened. Total processing time was at least 660 minutes. A microtome was used to cut 4-micrometer-thick tissue sections which were mounted on glass microscope slides. Slides from all 420 cases and the 40 controls were stained with HE. Prior to examination of the sections, the original laboratory number of each slide was covered with a blank label, thoroughly mixed on a table top and each allocated a new sequential index (Appendix 1; Column title: Random HE number).

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3.5.2.2 Examination of haematoxylin and eosin stained sections

Slides were examined by light microscopy (Olympus CX22 Binocular Microscope with 4x, 10x, 40x and 100x objectives and LED illumination). For each case, all the histomorphological features of the liver were systematically examined using a table (Appendix 3). The table included the entire range of microscopic lesions that are most often associated with RVFV infection. A second form (Appendix 4) was used to record a short summary of the pathology observed (if any) and the results.

Cases were classified as RVF positive or negative based on the presence or absence of characteristic lesions. Cases that were atypical or autolysed, but still suspicious for RVF, were classified as positive. The researcher was blinded to specimen origin and other test results. To determine if autolysis or tissue pigments influences the accuracy of the tests, the severity of autolysis was qualitatively graded as mild, moderate, or severe and the presence or absence of tissue pigments (i.e. acid haematin, haemosiderin, bilirubin, lipofuchsin) was recorded. Mild autolysis was defined as cases where morphological detail within all the cells and histological structures of the liver was distinct. Moderate autolysis denoted cases where cytoplasmic borders were still distinct, but some cells showed fading of nuclei and/or stained uniformly eosinophilic and putrefactive bacteria were present. Severe autolysis was defined as cases that showed complete loss of basophilia and where cells had indistinct cytoplasmic borders, nuclei had faded completely, all the red blood cells had lysed, and many putrefactive bacteria were present. It was believed that the age of foetuses might also influence the accuracy of the tests. The age of foetuses was not recorded by submitting officials and was subjectively estimated by noting the amount of extramedullary haematopoiesis. The severity of extramedullary haematopoiesis (EMH), if present, was graded as mild, moderate, or severe based on the estimated number of hematopoietic precursor cells per high power field (400x magnification). Mild EMH was defined as 1 to 50, moderate as 51 to 100 and severe as more than 100 hematopoietic precursor cells per high power field.

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The original laboratory numbers assigned by IDEXX or the Faculty were recorded against the randomly assigned number after all cases had been examined and results documented (Appendix 4). All randomly allocated numbers and results were subsequently recorded against the original laboratory number in an Excel spread sheet (Appendix 1).

3.5.3 Immunohistochemistry

3.5.3.1 Primary antibodies used for immunolabelling

Polyclonal hyperimmune mouse ascitic fluid to RVFV was used as primary antibody for the RVFV immunolabelling. The ascitic fluid was prepared by the South African National Institute for Communicable Diseases (NICD) in 1996 as described by Sartorelli *et al.* (1966) and Swanepoel *et al.* (1986) (Van Der Lugt *et al.* 1996, Sartorelli, Fischer & Downs 1966, Swanepoel *et al.* 1986). Similarly polyclonal hyperimmune mouse ascitic fluid, prepared by the NICD, was used as primary antibody for the WBV immunolabelling (Van Der Lugt *et al.* 1996, Sartorelli *et al.* 1966, Swanepoel *et al.* 1986). Rabbit polyclonal antiserum (Animal Health Trust, Newmarket, Suffolk, UK) raised against the Ab4 (neuropathogenic) strain of EHV-1 and monoclonal hyperimmune mouse ascitic fluid to bovine herpesvirus 1 (Veterinary Medical Research and Development, Pullman, Washington, USA) were used as the primary antibodies for the EHV-1 and BHV-1 immunolabelling respectively. The 420 field cases from the 2010 RVF outbreak were labelled with the antibody against RVFV. Twenty of the control specimens were labelled with antibody against RVFV while the remaining 20 were labelled with antibody against EHV-1, BHV-1, or WBV (Table 1).

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Table 1: Sections from the 420 field cases and 40 samples selected for randomization among the 2010 Rift Valley fever outbreak cases were variously labelled with relevant or irrelevant antibodies, as indicated, and randomly dispersed amongst the field cases.

Primary antibody used for IHC	Disease category with the number of cases tested with the primary antibody						
	2010 RVF	2009 RVF	Hepatotoxic plants	Salmonellosis	EHV-1	IBR	WBD
RVFV[1]	420	–	7*	1*	5*	2*	5*
EHV-1[2]	–	4*	–	–	5	–	–
BHV-1[3]	–	–	–	–	–	2	–
WBV[4]	–	4*	–	–	–	–	5

[1]Mouse polyclonal antiserum to Rift Valley fever virus. [2] Rabbit polyclonal antiserum to equine herpesvirus 1.

[3] Mouse monoclonal antiserum to bovine herpesvirus 1. [4] Mouse polyclonal antiserum to Wesselsbron virus.

* The Rift Valley fever virus antibody was an irrelevant antibody for all these cases

RVFV = Rift Valley fever virus. EHV-1 = Equine herpesvirus 1. BHV-1 = Bovine herpesvirus 1. WBV = Wesselsbron virus.

RVF = Rift Valley fever. IBR = Infectious bovine rhino-tracheitis. WBD = Wesselsbron disease

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Enzyme-linked immunosorbent assays (ELISA) were conducted on the RVFV antiserum, at the ARC-OVI, to determine the exact epitope affinity of the antibody. This was necessary to demonstrate conditional independence of rRT-PCR and IHC. Initially it was thought that the antibody detected the structural glycoproteins, G_N and G_C encoded by the M genomic segment of the virus. An indirect ELISA based on the recombinant nucleocapsid protein of RVFV (Paweska, Jansen van Vuren & Swanepoel 2007), however, gave negative results, disproving this assumption. The next possible targets for the antibody were proteins encoded by the small (S) segment of the virus, namely, the nucleoprotein (Np) or NSs (non-structural protein encoded by the S segment). NSs proteins form filamentous structures in infected hepatocytes that are visible under the standard light microscope as rod-shaped or oval eosinophilic intranuclear inclusions (Mansuroglu *et al.* 2010). This protein could be excluded as a target for the antibody since immunolabelling for RVFV is not associated with intranuclear inclusions. A second indirect ELISA based on the recombinant Np was carried out (Jansen van Vuren *et al.* 2007). This test yielded positive results demonstrating that the antibody detects epitopes on the RVFV Np.

3.5.3.2 Processing of immunolabelled sections

The same methodology for sectioning, mounting, deparaffinising and rehydration was employed for all sections irrespective of the target antigen. Four-micrometer-thick tissue sections were mounted on positively-charged microscope slides (SuperFrost® Plus, Menzel-Glasser®). The sections were then dried overnight in an incubator at 37 °C. Routine deparaffinising in xylol, followed by rehydration through a graded ethanol and distilled water series took place inside a fume hood (10 minutes in xylol, and 3 minutes each in 100 %, 96 %, and 70 % ethanol). Endogenous peroxidase activity was quenched by incubating sections with 3 % hydrogen peroxide in methanol for 15 minutes in a humidified chamber at room temperature (22 to 25 °C). This was followed by heat-induced epitope retrieval (HIER) or enzymatic digestion with Protease XIV (Table 2).

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Table 2: Antibody reagents, antigen retrieval and detection systems used in immunohistochemistry.

Antibody	Animal source	Dilution	Incubation	Pre-treatment	Detection system
RVFV	Mouse	1:500	30 min	HIER, Citrate, pH 6.0	ABC*
EHV-1	Rabbit	1:400	30 min	Protease XIV	ABC*
BHV-1	Mouse	1:2000	30 min	Protease XIV	ABC*
WBV	Mouse	1:500	20 min	HIER, TrisEDTA, pH 9.0	Polymer detection system

*Avidin-biotin complex (ABC)

RVFV = Rift Valley fever virus. EHV-1 = Equine herpesvirus 1.
BHV-1 = Bovine herpesvirus 1. WBV = Wesselsbron virus.
HIER = heat-induced epitope retrieval.

Epitopes for RVFV and WBV were recovered using the heat-induced epitope retrieval (HIER) method via microwave heat in different buffer solutions at 96 °C (citrate buffer solution at pH 6 for RVF and TrisEDTA buffer solution at pH 9 for WBV). For EHV-1 and BHV-1, epitope recovery was achieved through enzymatic digestion by immersing the slides in 50 mg of protease XIV (catalogue no: P5147-5G, Sigma Chemical Co, St. Louis, Missouri, USA) in 100 ml of PBS/BSA buffer solution (pH 7.6, pre-warmed to 37 °C) for 30 minutes at 37 °C in a humidified chamber. Sections were rinsed in distilled water, followed by 5 minutes in a 0.1 molar phosphate buffered saline (PBS), pH 7.6 containing 0.1 % bovine serum albumin (BSA).

For those cases where the target antigen was RVFV or BHV-1, non-specific epitopes were blocked on all the slides by pre-incubation with normal horse serum (catalogue no: H 0146, Sigma Chemical Co, St. Louis, Missouri, USA). Where the target was EHV-1, non-specific

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epitopes were blocked with normal goat serum (catalogue no: G9023, Sigma Chemical Co, St. Louis, Missouri, USA). For all three procedures the blocking serum was diluted 1:10 with PBS/BSA buffer, pH 7.6 and the slides incubated for 20 minutes in a humidified chamber at room temperature. Following blocking the serum was drained and replaced with 100 µl of the primary antibody (i.e. enough to completely cover the tissues), diluted in PBS/BSA buffer and incubated at 37 °C for 60 minutes in a humidified chamber. Optimal dilution for the RVFV, BHV-1 and EHV-1 sera in PBS/BSA buffer, that resulted in the best specific immunolabelling while providing the least background staining, were 1:500, 1:2000 and 1:400 respectively for each serum.

An avidin-biotin complex (ABC) immunoperoxidase technique was used to detect target antigens in sections treated with anti-RVFV, anti-BHV-1, and anti-EHV-1 serum. To detect RVFV and BHV-1 antigen biotinylated polyclonal rabbit-anti-mouse secondary or link antibody (catalogue no: E0432, DakoCytomation, Glostrup, Denmark) was used at a dilution of 1:500 in PBS/BSA buffer. To discourage nonspecific binding biotinylated polyclonal goat-anti-rabbit secondary or link antibody (catalogue no: E0432, DakoCytomation, Glostrup, Denmark) was used to detect EHV-1 instead of rabbit-anti-mouse secondary antibody. The secondary antibody was incubated with the tissue sections for 30 minutes at room temperature in a humidified chamber, followed by rinsing with distilled water and then PBS/BSA buffer as previously described. Peroxidase conjugated avidin, diluted as per kit instructions, (Vectastain ABC Kit, Elite PK6100 Standard, Vector Laboratories, Burlingame, California, USA) was applied and incubated on the sections for 30 minutes at room temperature in a humidified chamber. Sections were immersed in a NovaRED substrate (catalogue no: SK-4800, Vector Laboratories, Burlingame, California, USA), reconstituted according to manufacturer's instructions, using the droplet method for approximately 1-2 minutes at room temperature in a humidified chamber. Positive tissue control sections treated with the same substrate were examined for positive immunolabelling using light microscopy at 10X magnification. When there was clear evidence of positive

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immunolabelling in the positive control section, all sections in the batch were immediately rinsed in a distilled water bath to halt the substrate reaction. Sections were then counterstained with Mayer's haematoxylin for 3-4 minutes and rinsed with tap water, at gentle bathing pressure, for 10 minutes to remove excess substrate. Sections were then routinely dehydrated through increasing alcohol concentrations and xylol, mounted using entelan and then coverslipped.

A polymer detection system was used (catalogue no: RE7150-K: NovoLink™ Min Polymer Detection System, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) was used to visualize WBV antigen. Slides were incubated for 5 minutes with the protein block supplied by the manufacturer after completions of sectioning, mounting, deparaffinising, rehydration, neutralizing of endogenous peroxidase activity and antigen retrieval. Sections were rinsed in distilled water and then in PBS/BSA buffer for 5 minutes. This was followed by incubation with 100 µl of the primary antibody at a dilution of 1:500 in PBS/BSA buffer for 60 minutes at 37 °C in a humidified chamber. Slides were then incubated with the post primary block for 20 minutes, rinsed as described previously and then incubated with the NovoLink polymer for a further 20 minutes. Sections were again rinsed and immersed in a NovaRED substrate and further processed as previously described.

Immunolabelled sections were randomised independently from the HE sections using the same procedure. Therefore the random numbers allocated to the immunolabelled sections were different from the counterpart HE stained section (Appendix 1; Column title: Random IHC number). This was performed to reduce bias in the reading of the IHC slides since pathologists can often relate the pattern of lesions observed in an immunolabelled section back to the HE stained section.

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3.5.4 Examination of immunolabelled sections

Slides were examined by light microscopy (Olympus CX22 Binocular Microscope with 4x, 10x, 40x and 100x objectives and LED illumination). For each case, all the morphological features of the liver were systematically examined using a table (Appendix 5). The table included all variations in immunolabelling that were expected to be associated with RVFV infection. A second form (Appendix 6) was used to record results. Comments were added if deemed useful for future reference (e.g. if immunolabelling was noted in other organs).

The original laboratory numbers assigned by IDEXX or the Faculty were recorded against the randomly assigned number after all the cases had been examined and results documented (Appendix 6). All randomly allocated numbers and results were subsequently linked to the original laboratory number in an Excel spread sheet (Appendix 1).

3.6 Sensitivity and specificity analysis

In the absence of a gold standard or prior knowledge concerning the true RVFV infection status of the population, sensitivity (Se) and specificity (Sp) of rRT-PCR, histopathology, and IHC could not be estimated directly (Joseph *et al.* 1995). Instead, diagnostic accuracy of the tests was estimated in a latent class analysis using a Bayesian framework (Branscum *et al.* 2005, Enøe *et al.* 2000, Joseph *et al.* 1995, Scott *et al.* 2007, Stalker & Hayes 2007). In this study the available information included the number of cattle and sheep (goats were excluded) divided into age groups of adults, neonates and foetuses with each of the 8 possible combinations of positive and negative test results (e.g. positive or negative for rRT-PCR, histopathology or IHC). The total count for the test pattern was the sum of the unobserved (latent) number of infected and uninfected animals for each category. For example, 30 of the 71 adult bovine animals tested positive for all three tests and 23 tested negative for all three tests. The basic statistical model was constructed for three conditionally independent tests and a single population (Branscum *et al.* 2005). Conditional independence for the three tests was assumed since they are each based on a different

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biological principle (Toft *et al.* 2005). Histopathology relies on the observation of histological lesions using light microscopy. The antibody used in the immunoperoxidase technique detects epitopes on the RVFV nucleoprotein, as opposed to PCR that detects conserved sequences on the M segment of the viral genome.

Beta prior probability distributions were elicited for unknown parameters (Table 3). A non-informative prior probability (i.e. uniform distribution in which all values between 0 and 1 are equally likely) was used to model prevalence since data concerning sample prevalence was not available. Sensitivity and Sp prior values for rRT-PCR were modelled according to the previous experience of a co-investigator (Dr Marco Romito) with expertise using this assay. The most probable value for Se was determined to be 0.98 (assumed to be the median), while the expert was 95% sure that it was at least 0.95. The most probable Sp value of rRT-PCR during an epidemic was considered to be 0.78 and it was thought to be at least 0.60 with 95% certainty. Published information concerning the diagnostic accuracy of histopathology and IHC is limited. Therefore, the prior probability distribution for Se and Sp of histopathology was extrapolated from the results obtained from the 40 control cases. The most probable value for Se was determined to be 0.88 (7/8) and alpha and beta of the distribution were adjusted to equal the sample size (n=8) forming the basis for this estimation. The prior distribution for Sp was based on an estimate of 0.86 (18/22) and the alpha and beta adjusted based on the sample size (n=22). Immunohistochemistry correctly classified all control cases and therefore, non-informative prior probabilities were employed.

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Table 3: Beta prior probability distributions for unknown parameters in the Bayesian model used to estimate the diagnostic test accuracy and prevalence of real-time reverse transcription-polymerase chain reaction, histopathology, and immunohistochemistry to detect Rift Valley fever virus in liver specimens.

Population and test	Parameter	Prior probability distribution (β)	Median	90% credibilit intervals
Sample population	Prevalence	1.0,1.0*	0.50	0.50, 0.95
rRT-PCR	Sensitivity	107,2.5	0.98	0.95, 0.99
rRT-PCR	Specificity	12,3.3	0.78	0.60, 0.93
Histopathology	Sensitivity	6.9,1.1	0.89	0.63, 0.99
Histopathology	Specificity	18.45,3.55	0.85	0.70, 0.95
Immunohistochemistry	Sensitivity	1.0,1.0*	0.50	0.50, 0.95
Immunohistochemistry	Specificity	1.0,1.0*	0.50	0.50, 0.95

* Uniform (non-informative) prior probability distribution in which all values between 0 and 1 are equally likely.
rRT-PCR = Real-time reverse-transcriptase polymerase chain reaction.

Diagnostic test accuracy was estimated by Markov chain Monte Carlo methods, utilizing available statistical software (WinBUGS Version 1.4, MRC Biostatistics Unit, Cambridge, UK). Program code for the conditional independence model using informative priors for rRT-PCR and histopathology and uninformative priors for prevalence and IHC is provided in Appendix 7. Analyses were performed on the cross-classified test outcomes (Appendix 8) for rRT-PCR, histopathology, and IHC in respect to the unstratified data set, and the data stratified into species (cattle or sheep), age category (adult, neonate or foetus), level of autolysis (mild, moderate or severe) and the presence or absence of tissue pigments (haemosiderin, bilirubin or lipofuchsin). The informative priors for rRT-PCR and

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histopathology was kept the same in the program code used for the unstratified and the stratified analyses model (Appendix 7), whereas, the list following #data in the code was modified in each stratum to reflect the population in that stratum and each of the 8 possible combinations of positive and negative test results in that stratum (Table 4). The first 100,000 iterations were discarded as the burn-in and inferences were made based on the subsequent 20,000 iterations (based on two sampling chains). These were the posterior values obtained for all the parameters at a starting point of 100000 and sampled at point 40002 (Appendix 9: Posterior values obtained for each parameter for the data set; last 8 rows of each table).

Convergence between two chains was assessed using the Gelman-Rubin statistic available in the software (Appendix 9). Autocorrelation among iterate values was assessed and only every fifth value was retained to reduce the impact of this correlation (Appendix 9). Medians and percentiles (2.5^{th} to 97.5^{th}) of the posterior distributions were used as the point estimates and credibility intervals (CI), respectively.

3.7 Sensitivity analysis

A sensitivity analysis was conducted employing non-informative prior probability distributions (Appendix 10). This included a sensitivity analysis of the data set using: an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC (Appendix 10.1); an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC (Appendix 10.2); and uninformative priors for prevalence, rRTPCR, histopathology, and IHC (Appendix 10.3).

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Table 4: Modifications made to the list following #data in the programme code in each stratum, to reflect the population in that stratum, and each of the 8 possible combinations of positive and negative test results in that stratum.

	List used following #data in the program code
Sample population	list(n1=380,y1=c(188,8,11,50,6,9,0,124))
Species	
Bovine	list(n1=119,y1=c(57,1,2,19,2,4,0,34))
Ovine	list(n1=261,y1=c(131,7,9,31,4,5,0,74))
Age	
Adult	list(n1=201,y1=c(87,6,6,30,3,3,0,66))
Neonate	list(n1=85,y1=c(45,0,2,14,2,1,0,21))
Foetus	list(n1=94,y1=c(56,2,3,6,1,5,0,21))
Autolysis	
Mild	list(n1=243,y1=c(127,2,6,24,3,6,0,75))
Moderate	list(n1=72,y1=c(41,0,3,7,2,1,0,18))
Severe	list(n1=65,y1=c(20,6,2,19,1,2,0,15))
Tissue pigments	
Absent	list(n1=280,y1=c(138,5,8,37,5,5,0,82))
Present	list(n1=100,y1=c(50,3,3,13,1,4,0,26))

n1 = population in that stratum

c = the 8 possible combinations of positive and negative test results in that stratum

3.8 Estimating repeatability of histopathology and immunohistochemistry

A second reader evaluated 84 cases (168 slides) where the test result pattern for rRT-PCR, histopathology, and IHC were not in perfect agreement (i.e. results for the three tests were not all positive or all negative). The assumption was made that the second reader would obtain the same result for all those cases where all the test results obtained by the first

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reader were in perfect agreement (all results for all three tests were positive or negative respectively). This assumption is reasonable since uncomplicated cases of Rift Valley fever show very characteristic histological lesions (Coetzer 1977, Daubney *et al.* 1931, Easterday *et al.* 1962a, Easterday *et al.* 1962b) and positive immunolabelling is recognised without any difficulty (Van Der Lugt *et al.* 1996). A qualitative assessment of 120 histopathology and immunohistochemistry slides (those randomly numbered from 1 to 60) was made by both readers to evaluate this assumption.

Results were dichotomized into positive or negative and cross tabulated (Appendix 11 and 12). Cohen's kappa coefficient was calculated using the freeware program OpenEpi (Version 3, Open Source Calculator, Diagnostic Test). Unstratified and stratified analyses were performed in respect to species (cattle or sheep), age category (adult, neonate or foetus), level of autolysis (mild, moderate or severe) and the presence or absence of tissue pigments (haemosiderin, bilirubin or lipofuchsin).

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CHAPTER 4

RESULTS

4.1 Descriptive results

A total of 420 animals from the 2010 RVF outbreak were selected for study and included 127 cattle (73 adults, 21 neonates, 33 foetuses), 273 sheep (137 adults, 68 neonates, 68 foetuses) and 20 goats (11 adults, 2 neonates, 7 foetuses). The goats were excluded from the sensitivity and specificity analysis of the data set since 4 of the goats tested positive on all three tests and the other 16 tested negative on all tests. Also, the number of goats available to study was too small to make any useful inferences about the use of the tests in this species. A further 20 cases (14 from sheep and 8 from cattle) were excluded from the statistical analysis because liver specimens were absent or not accurately identifiable in the HE stained sections or immunolabelled sections. Ultimately, therefore, the statistical analysis was conducted for a total of 380 animals that included 119 cattle (71 adults, 20 neonates, 28 foetuses) and 261 sheep (130 adults, 65 neonates, 66 foetuses).

Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the unstratified data set and the data stratified into species (cattle or sheep), age category (adult, neonate or foetus), level of autolysis (mild, moderate or severe) and the presence or absence of tissue pigments are presented in Appendix 8. For the unstratified data set, 49% percent of the cases (188/380) were positive on rRT-PCR, histopathology, and IHC whereas 33% (124/380) were negative on all assays (Appendix 8.1). Sixty-six percent (79/119) of cattle were positive on rRT-PCR, 54% (64/119) on histopathology, and 51% (61/119) on IHC (Appendix 8.2). For the sampled sheep, percentages positive on rRT-PCR, histopathology and IHC were 68% (178/261), 56% (147/261) and 55% (144/261) respectively (Appendix 8.3). Sixty-four percent (129/201) of the adult ovine and bovine animals tested positive on rRT-PCR, 50% (101/201) on histopathology, and 48% (96/201) on IHC (Appendix 8.4).

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Seventy-two percent (61/85) of the ovine and bovine neonates tested positive on rRT-PCR, 56% (48/85) on histopathology, and 58% (49/85) on IHC (Appendix 8.5). For the sampled foetuses, percentages positive on rRT-PCR, histopathology and IHC were 71% (67/94), 68% (64/94) and 64% (60/94) respectively (Appendix 8.6).

The model where uninformative priors were used for prevalence and IHC, and informative priors for rRT-PCR and histopathology, estimated prevalence in the sample population as 52.7% (95% CI of 47.7 to 57.8%) (Table 5). Stratified analysis suggested a slightly higher prevalence in sampled sheep (56.8% with a CI of 50.5% - 62.9%) compared to cattle (51.1% with a CI of 42.1% - 60.1%). Prevalence was 49.6% (CI of 42.4% - 56.8%) in adult animals, 56.9% (CI of 46.3 - 67.3%) in neonates, and 64.5% (CI of 54.2% - 73.9%) in foetuses (Table 5).

Eighty eight percent (35/40) of the control specimens stained with HE were correctly classified. Lesions detected in the cases of plant hepatotoxicosis, EHV-1, and salmonellosis were correctly identified as inconsistent with RVFV infection. Histopathology incorrectly classified 3 of the 4 IBR cases and 1 of the 10 WBD cases as positive for RVFV infection. One of the 2009 RVF cases was incorrectly classified as WBD. Immunohistochemistry correctly classified all of the control cases.

For the sampled foetuses, percentages positive or negative on rRT-PCR, histopathology and IHC were 60% (56/94) and 22% (21/94) respectively. This represented 82% percent (77/94) of all the foetuses. Eighty percent (12/15), 68% (15/22), 83% (31/37) and 95% (19/20) of the foetuses classified as showing no, mild, moderate or severe EMH respectively, were classified as either positive on rRT-PCR, histopathology and IHC, or negative on all three tests.

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Table 5: Estimates of Rift Valley fever diagnostic test accuracy and prevalence in cattle and sheep in which the true infection status was unknown, as determined by using Bayesian latent class methods. The unstratified sample population and stratified populations were independently modelled and analysed to allow for the estimation of stratum specific values of diagnostic test accuracy.

		rRT-PCR		Histopathology		IHC	
	Prevalence	**Se**	**Sp**	**Se**	**Sp**	**Se**	**Sp**
Sample population	0.53 [0.48, 0.58]	0.97 [0.95, 0.99]	0.72 [0.65, 0.78]	0.95 [0.91, 0.97]	0.92 [0.88, 0.96]	0.98 [0.94, 0.99]	0.99 [0.97, 1.00]
Species							
Bovine	0.51 [0.42, 0.60]	0.98 [0.95, 0.99]	0.68 [0.57, 0.78]	0.96 [0.90, 0.99]	0.90 [0.82, 0.95]	0.98 [0.92, 0.99]	0.98 [0.91, 0.99]
Ovine	0.57 [0.50, 0.63]	0.98 [0.95, 0.99]	0.71 [0.63, 0.79]	0.94 [0.89, 0.97]	0.92 [0.86, 0.96]	0.96 [0.91, 0.99]	0.99 [0.95, 0.99]
Age							
Adult	0.50 [0.42, 0.57]	0.98 [0.95, 0.99]	0.70 [0.61, 0.78]	0.94 [0.88, 0.98]	0.93 [0.87, 0.97]	0.95 [0.88, 0.99]	0.99 [0.94, 0.99]
Neonate	0.57 [0.46, 0.67]	0.97 [0.94, 0.99]	0.66 [0.52, 0.78]	0.96 [0.88, 0.99]	0.92 [0.84, 0.97]	0.99 [0.92, 0.99]	0.97 [0.86, 0.99]
Foetus	0.65 [0.54, 0.74]	0.98 [0.95, 0.99]	0.79 [0.66, 0.90]	0.95 [0.87, 0.99]	0.83 [0.71, 0.92]	0.97 [0.89, 0.99]	0.97 [0.85, 0.99]
Autolysis							
Mild	0.56 [0.50, 0.62]	0.98 [0.96, 0.99]	0.77 [0.68, 0.84]	0.95 [0.91, 0.98]	0.92 [0.86, 0.96]	0.99 [0.96, 0.99]	0.99 [0.95, 0.99]
Moderate	0.63 [0.51, 0.74]	0.97 [0.94, 0.99]	0.75 [0.60, 0.87]	0.93 [0.84, 0.99]	0.91 [0.80, 0.97]	0.98 [0.92, 0.99]	0.96 [0.81, 0.99]
Severe	0.41 [0.27, 0.56]	0.98 [0.94, 0.99]	0.54 [0.40, 0.69]	0.92 [0.77, 0.99]	0.87 [0.76, 0.95]	0.81 [0.61, 0.98]	0.96 [0.85, 0.99]
Tissue pigments							
Absent	0.55 [0.49, 0.61]	0.97 [0.95, 0.99]	0.70 [0.62, 0.77]	0.95 [0.90, 0.98]	0.93 [0.87, 0.97]	0.98 [0.94, 0.99]	0.99 [0.95, 0.99]
Present	0.55 [0.45, 0.65]	0.98 [0.95, 0.99]	0.70 [0.58, 0.81]	0.94 [0.86, 0.99]	0.87 [0.77, 0.94]	0.96 [0.87, 0.99]	0.97 [0.88, 0.99]

rRT-PCR = Real-time reverse-transcriptase polymerase chain reaction. IHC = Immunohistochemistry. Se = sensitivity. Sp = specificity.

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Table 6: Results for the sensitivity analysis.

Uninformative priors used in the analysis	Pr	rRT-PCR		Histopathology		IHC	
		Se	Sp	Se	Sp	Se	Sp
Pr and IHC	0.53 [0.48, 0.58]	0.97 [0.95, 0.99]	0.72 [0.65, 0.78]	0.95 [0.91, 0.97]	0.92 [0.88, 0.96]	0.98 [0.94, 0.99]	0.99 [0.97, 1.00]
Pr, rRT-PCR and IHC	0.53 [0.48, 0.58]	0.97 [0.94, 0.99]	0.71 [0.64, 0.77]	0.95 [0.91, 0.97]	0.92 [0.88, 0.96]	0.98 [0.94, 1.00]	0.99 [0.97, 1.00]
Pr, histopathology, and IHC	0.53 [0.48, 0.58]	0.97 [0.95, 0.99]	0.72 [0.65, 0.78]	0.95 [0.91, 0.97]	0.93 [0.88, 0.97]	0.97 [0.94, 1.00]	0.99 [0.97, 1.00]
Pr, rRT-PCR, IHC and histopathology	0.50 [0.43, 0.57]	0.90 [0.24, 0.98]	0.54 [0.02, 0.76]	0.85 [0.04, 0.97]	0.82 [0.03, 0.96]	0.90 [0.00, 1.00]	0.92 [0.00, 1.00]

Se = Sensitivity. Sp = Specificity.

Pr = Prevalence. rRT-PCR = Reverse-transcriptase polymerase chain reaction. IHC = Immunohistochemistry.

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4.2 Sensitivity analysis

Independent models for the sensitivity analysis with non-informative priors for prevalence, rRT-PCR and IHC; and then prevalence, histopathology and IHC were both able to converge (Table 6 and Appendix 10.1 and 10.2). Estimates from these models for prevalence, Se and Sp varied by 1% or less compared to results obtained when uninformative priors were used for prevalence and IHC (Table 6). The model with non-informative priors for all unknown parameters would not converge (Table 6 and Appendix 10.3).

4.3 Real-time reverse-transcriptase polymerase chain reaction

The Se and Sp of rRT-PCR were 97.4% (95% CI, 95.2% - 98.8%) and 71.7% (95% CI, 65.0% - 77.9%) respectively. Stratified analysis suggested that the Se of rRT-PCR was consistent over evaluated categories (varied by 1 to 2% relative to the Se estimate for the sample population). Specificity was slightly more variable with the lowest value in severely autolysed specimens (54.3% with 95% CI of 40.3% - 68.5%). Values for Sp in the age and tissue pigment strata varied by only 1 to 2% relative to the values obtained for the sample population.

4.4 Histopathology

The Se of histopathology for the entire sample population was 94.6% (95% CI, 91% - 97.2%). Sensitivity also remained within a narrow limit in all evaluated strata and only varied by 1 to 3% relative to the overall Se estimate.

Generally, the morphology of the lesions was very distinctive. Lesions were similar in cattle and sheep but the extent of liver involvement varied with age. In adult animals, foci of hepatocellular death were random (Fig. 1) and could be multifocal, focally extensive, or confluent bridging. Neonates and foetuses tended to present with cell death involving almost all hepatocytes (Fig 5 and 9). Liver injury was generally accompanied by haemorrhage and a mild to moderate infiltrate of neutrophils, lymphocytes, and Kupffer cells

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(Fig. 2 and 3). Scattered hyperplastic Kupffer cells were present within the sinusoids. A mild infiltrate of mononuclear inflammatory cells in the portal tracts (Fig. 3), and involvement of hepatocytes bordering the portal tract (Fig. 1) was another common finding. Affected hepatocytes displayed features of apoptosis (Fig. 4 and 10) that included dissociation of cells, with shrinkage and rounding, hypereosinophilic cytoplasm and karyorrhexis or karyolysis. Apoptotic bodies were identified as small cytoplasmic fragments (Fig. 4). Neonates especially, often showed proliferating limiting plate epithelium (Fig. 5). Destruction of the portal triads, bile ducts, or proliferating limiting plate epithelium was not observed. Surviving hepatocytes usually had varying degrees of hydropic and/or fatty vacuolation and anisokaryosis (Fig. 4). A very distinctive feature in many cases (especially in neonates and foetuses) was randomly located foci of well circumscribed cytolysis (primary foci) (Fig. 6, 7 and 9). These foci varied in size and number and contained dense aggregates of hepatocellular debris, degenerate neutrophils, and scarce hyperplastic Kupffer cells. Rods to oval-shaped or round eosinophilic intranuclear inclusions were occasionally observed in injured hepatocytes (Fig 8 and 12). However, inclusion bodies were often difficult to detect and less common in the livers of adult animals compared to foetuses and neonates.

The Sp of histopathology was 92.3% (95% CI, 87.6% - 95.8%) overall and slightly more variable among strata with the lowest value in foetuses (83.0% with 95% CI of 71.3% - 91.6%). Lesions in some foetuses were more subtle, and included cellular dropout, scattered small round, disassociated cells, and marginated chromatin in the nuclei (Fig 11 and 12). The absence of typical eosinophilic intranuclear inclusions or foci of intense cytolysis in some cases made classification difficult.

Severe autolysis and the presence of tissue pigments had minor influences on the Sp of histopathology causing a decrease of 4.9% (from 92.3% to 87.4%) and 5.5% (from 92.3% to 86.8%) respectively. Autolysis obscured morphological details within the cells and histological structures in the liver so that cell injury could not always be distinguished from autolysis. Areas of hepatocellular death, however, usually contrasted sufficiently with areas

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where the architecture of the liver was not significantly disturbed (Fig. 25 and 26), so that a diagnosis of suspect (classified as positive in this study) was warranted in many severely autolysed cases.

Contrary to autolysis, the finding that the excessive tissue pigments such as bilirubin or haemosiderin, influenced the Sp of histopathology causing a decrease of 5.5% (from 92.3% to 86.8%) compared to the value obtained for the population was unexpected and there is no plausible explanation for this.

4.5 Immunohistochemistry

The overall Se and Sp of IHC was 97.6% (95% CI, 93.9% - 99.8%) and 99.4% (95% CI, 96.9% - 100%) respectively. With the exception of specimens with severe autolysis, both Se and Sp of IHC remained within a narrow limit in all strata and only varied by 1 to 4% relative to the Se estimate for the entire sample population.

Immunohistochemistry results indicated that hepatocytes are the predominant target of RVFV infection in the liver. In adult animals labelling was observed either throughout an area of hepatocellular injury (Fig. 13) or at the edges of lesions (Fig 14 and 15). Areas of hepatocellular death seem to become devoid of immunolabelling with lesion maturity (Fig. 14, 15 and 16). In neonates and foetuses diffuse positive immunolabelling suggested that virtually every hepatocyte contained Rift Valley fever viral antigens (Fig. 18 and 21) Positive immunolabelling was more difficult to detect in some foetuses (Fig. 22)

Fine diffuse to coarse granular labelling was observed in the cytoplasm of apoptotic hepatocytes (Fig. 16, 20, 23 and 24), and immunolabelled cytoplasmic fragments were frequently detected within the sinusoids and central veins (Fig. 24). Positively labelled Kupffer cells and degenerate neutrophils were sparse and convincing labelling of endothelial cells could not be detected. Furthermore, positive immunolabelling was not observed within the portal tract and, bile duct epithelial cells or cells proliferating within the limiting plate

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(Fig. 17 and 18). Positive labelling was largely absent or sparse, and usually peripherally located, in primary foci of hepatocellular death (Fig. 18, 19 and 23).

Immunolabelling was not detected in nuclei, remnants of nuclei or in association with intranuclear inclusion bodies (Fig. 24). Sensitivity decreased to 81.5% (95% CI, 60.1% - 97.5%) when specimens were severely autolysed. Sixty-five cases were classified as severely autolysed on histological examination. Twenty nine of these were classified as positive for RVFV infection on histopathology and 23 cases demonstrated positive immunolabelling that varied from sparse to widespread (Fig. 27 and 28).

At least two cases were detected where two or more sections from the liver were examined revealing strong positive immunolabelling in one section with typical histological lesions but no lesions or positive immunolabelling in other sections.

4.6 Estimating repeatability of histopathology and immunohistochemistry

κ-values obtained for the 84 cases where the test results for rRT-PCR, histopathology and IHC were not in perfect agreement (Table 7) suggested anomalies with foetuses, severe autolysis and pigments. When the κ-values were calculated by factoring in test results for rRT-PCR, histopathology, and IHC that were all in perfect agreement (i.e. those obtained by the principle investigator) the differences in the κ-values obtained for foetuses, severe autolysis, and pigments as opposed to other categories was more pronounced. (Table 8).

Inter-rater agreement for histopathology decreased substantially from 0.91 in adult animals to 0.74 in foetuses suggesting that foetuses are more likely to be misclassified (Table 8). Inter-rater agreement also decreased substantially when specimens were severely autolysed: 0.90 for mild to moderate autolysis and 0.71 for severe autolysis. This result was in agreement with the qualitative assessment of 60 histopathology slides (those randomly numbered from 1 to 60) by both readers. The presence of tissue pigments also influenced the decision of the reader to classify cases as either consistent with RVFV infection or not,

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Plate 1

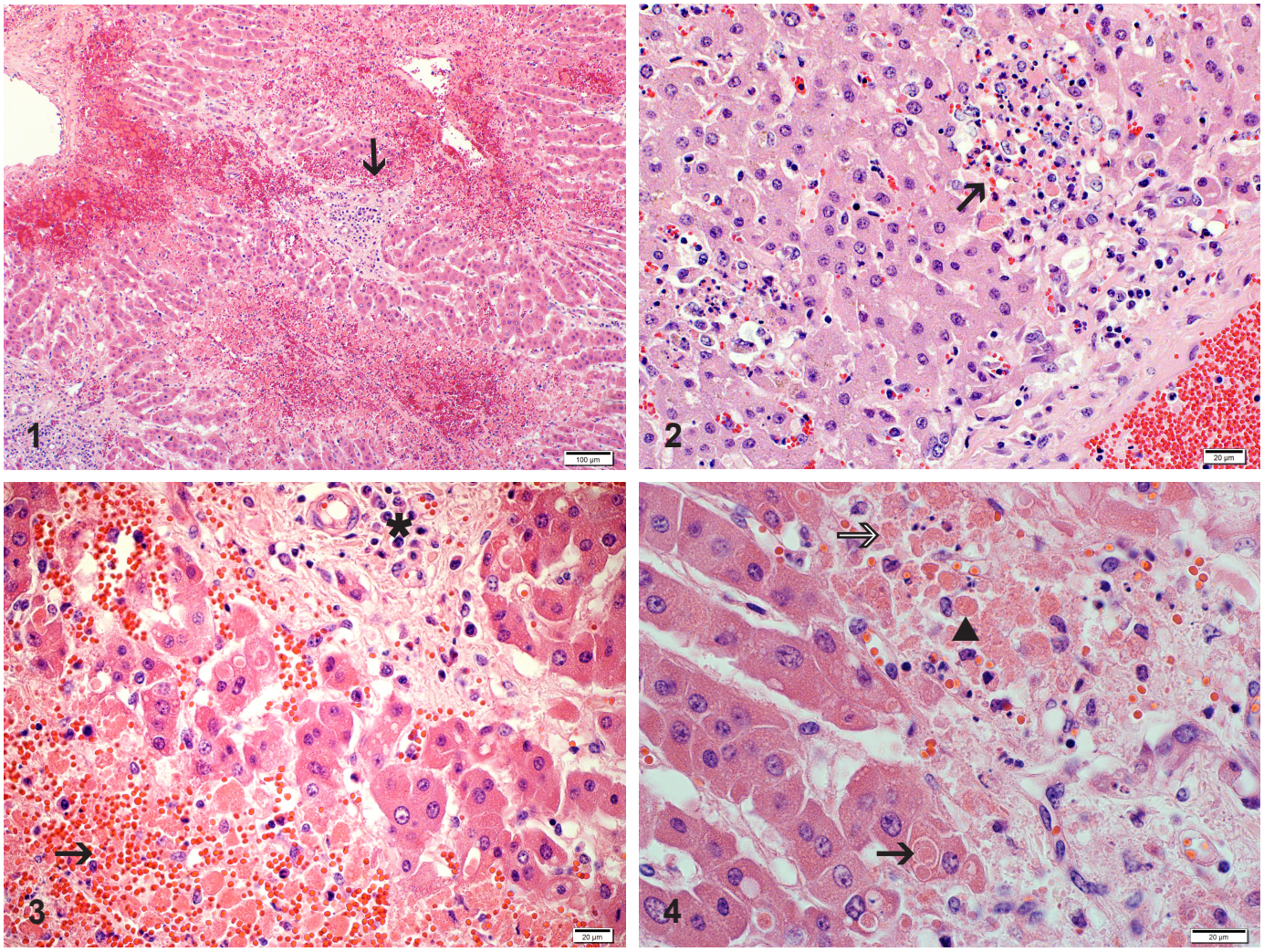


PLATE 1: Haematoxylin and eosin (HE)-stained sections showing lesions typical for Rift Valley fever virus infection in the liver of adult cattle and sheep.

FIG. 1: Adult bovine (case 390). Random multifocal areas of hepatocellular death and haemorrhage with involvement of hepatocytes within the limiting plate (black arrow). Bar = 100 µm.

FIG. 2: Adult ovine (case 5). A focus of hepatocellular death (black arrow) that contains apoptotic cells, intensely basophilic nuclear debris, and reactive Kupffer cells. Bar = 20 µm.

FIG. 3: Adult bovine (case 390). An area of hepatocellular death and haemorrhage (black arrow) is bordered by surviving hepatocytes in the periportal zone. The mild inflammatory reaction in evidence is characterized by scattered mononuclear inflammatory cells in the portal tract (asterisk), and a mixed infiltrate of neutrophils, lymphocytes, and reactive Kupffer cells amongst the apoptotic cells. Bar = 20 µm.

FIG. 4: Adult bovine (case 390). Apoptotic cells (black arrowhead) are disassociated, shrunken, and rounded, with hypereosinophilic cytoplasm. Some of the cells contain intensely eosinophilic hyaline globular masses (black arrow) or are fragmented into many small acidophilic bodies (open arrow). Surviving hepatocytes show mild hydropic change and anisokaryosis. Bar = 20 µm.

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Plate 2

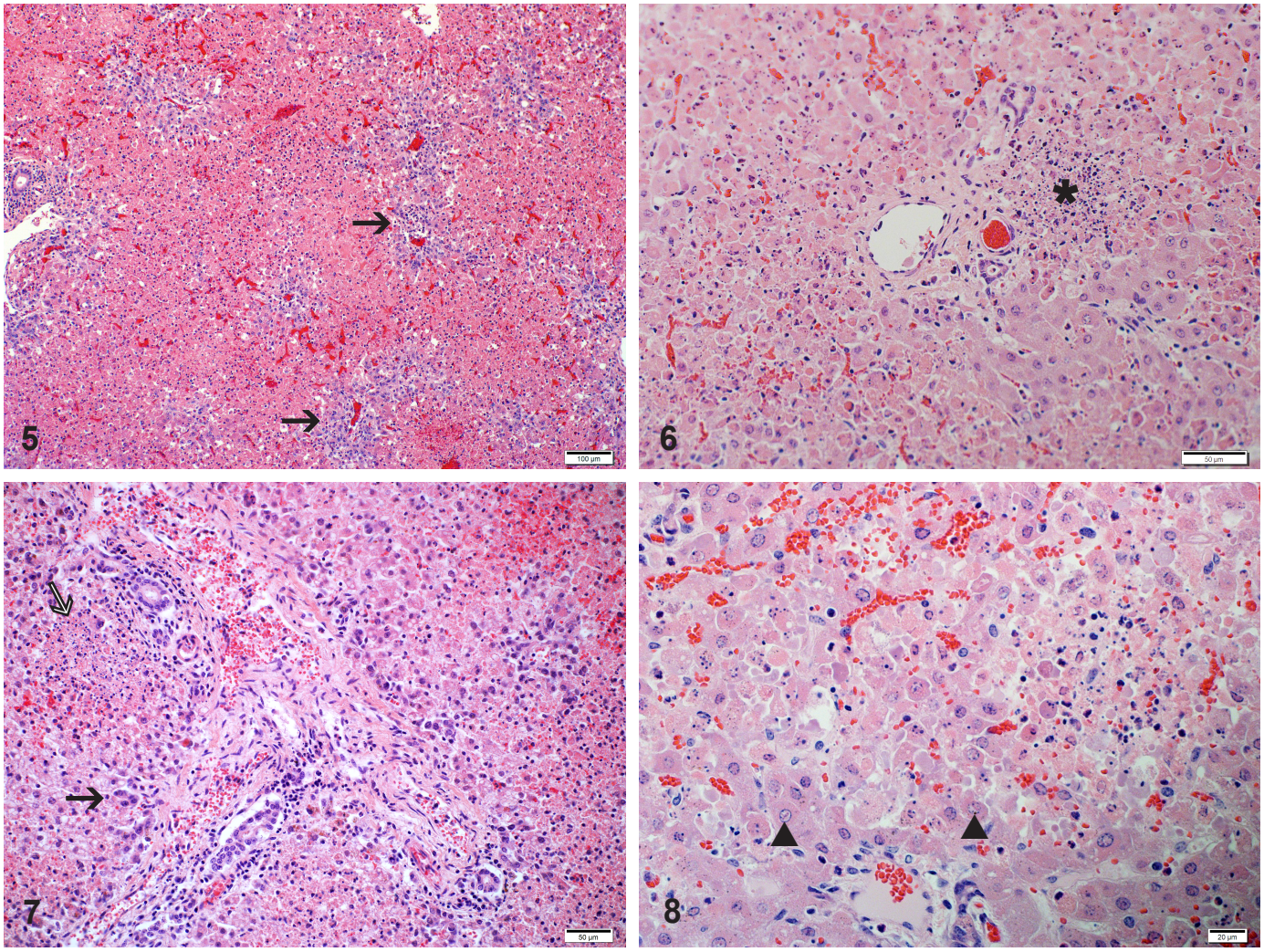


PLATE 2: Haematoxylin and eosin (HE)-stained sections showing lesions typical for Rift Valley fever virus infection in the liver of ovine and bovine neonates.

FIG. 5: Bovine neonate (case 306). Proliferating limiting plate epithelial cells (black arrows) and cell death involving all hepatocytes. Bar = 100 µm.

FIG. 6: Ovine neonate (case 60). Primary focus of hepatocellular death (asterisk) involving the limiting plate that contains a dense aggregate of cellular debris. Bar = 50 µm.

FIG. 7: Bovine neonate (case 14). Cell death involving virtually all hepatocytes, with mild limiting plate epithelial proliferation (black arrow) and a primary focus of hepatocellular death involving the limiting plate (open arrow). Bar = 50 µm.

FIG. 8: Ovine neonate (case 42). Hepatocytes with filamentous intranuclear inclusion bodies (black arrowheads). Bar = 20 µm.

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Plate 3

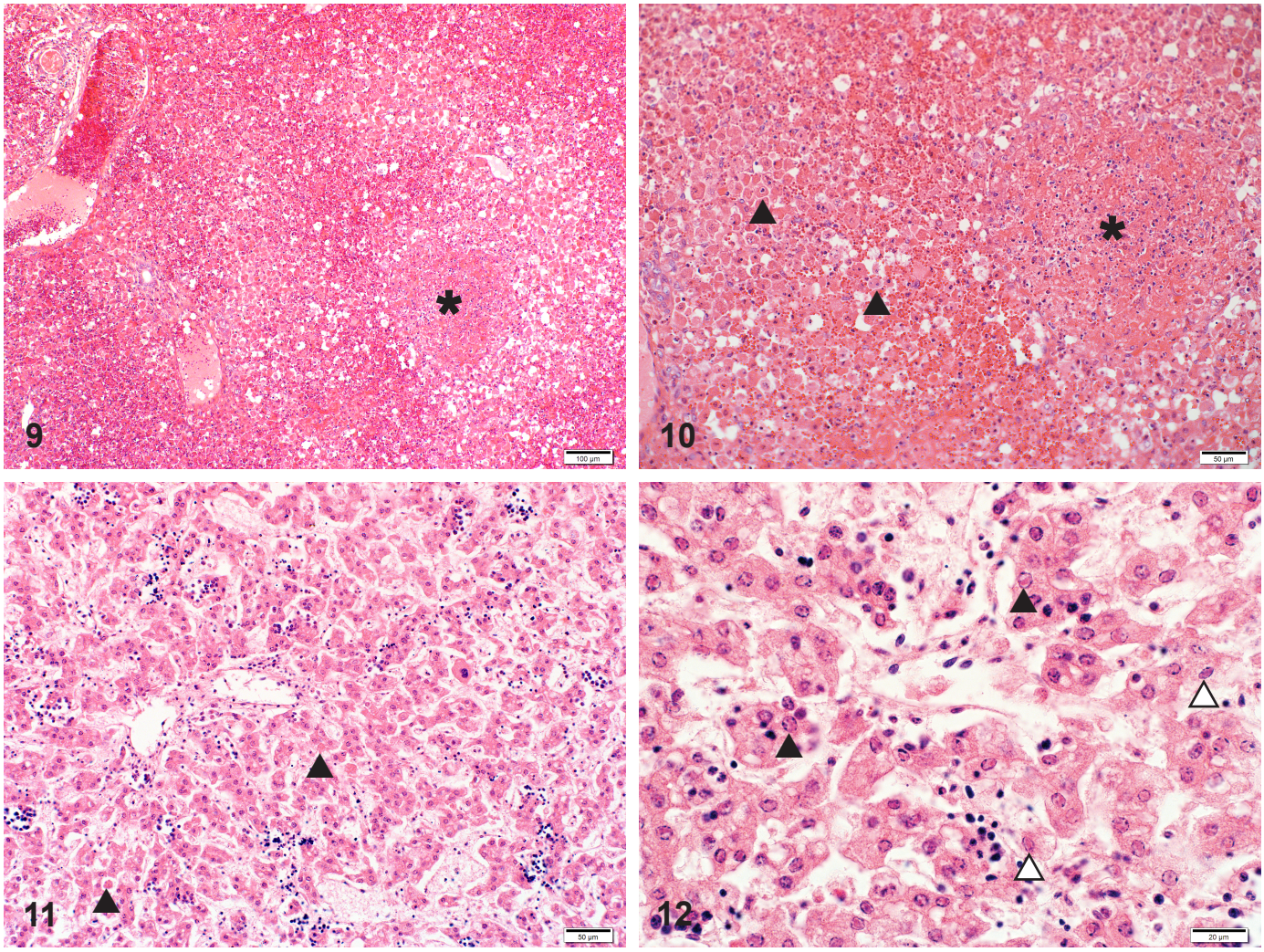


PLATE 3: Haematoxylin and eosin (HE)-stained sections showing lesions typical for Rift Valley fever virus infection in the liver of ovine and bovine foetuses.

FIG. 9: Ovine foetus (case 39). Cell death involving all hepatocytes and a well circumscribed primary focus of hepatocellular death (asterisk). Prominent cellular dropout gives the liver a moth-eaten appearance. Bar = 100 µm.

FIG. 10: Ovine foetus (case 39). Primary focus (asterisk) consisting of a dense aggregate of hepatocellular debris. Apoptotic cells (black arrowheads) are disassociated, shrunken, and rounded, with hypereosinophilic cytoplasm. Bar = 50 µm.

FIG. 11: Ovine foetus (case 199). Moderate extramedullary haematopoiesis and mild autolysis in a young foetus with subtle lesions. Haemorrhage is absent and apoptotic hepatocellular death, identifiable as scattered small round, disassociated cells, is inconspicuous (black arrowheads). Bar = 50 µm.

FIG. 12: Ovine foetus (case 199). Virtually every hepatocyte nucleus has marginated chromatin and contains rod to oval-shaped or round eosinophilic intranuclear inclusions (black arrowheads). Inclusions cause many of the nuclei to become oval shaped (white arrowheads). Cell injury and death is difficult to characterize due to the presence of mild autolysis. Bar = 20 µm.

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Plate 4

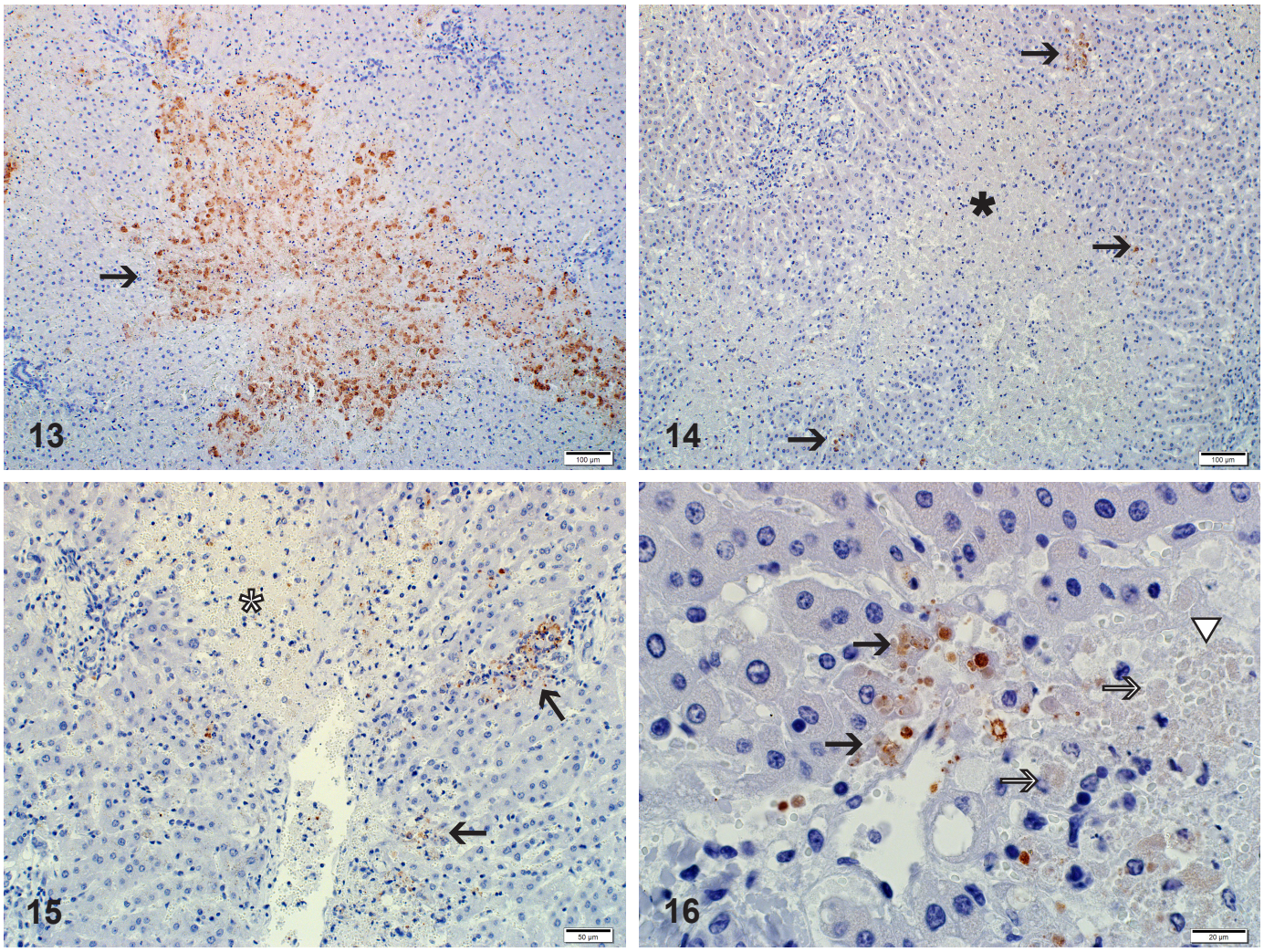


PLATE 4: Rift Valley fever virus antigen labelling in the liver of adult cattle and sheep.

FIG. 13: Adult ovine (case 447). Widespread red-brown cytoplasmic labelling throughout an area of hepatocellular injury (black arrow). Bar = 100 µm.

FIG. 14: Adult bovine (case 290). Sparse cytoplasmic labelling (black arrows) associated with occasional small groups of hepatocytes at the periphery of areas of hepatocellular death (asterisk). Bar = 100 µm.

FIG. 15: Adult ovine (case 5). Small foci of cytoplasmic labelling (black arrows) at the periphery of an area of hepatocellular death (asterisk). Bar = 50 µm.

FIG. 16: Adult bovine (case 290). Coarse red-brown granular labelling in the cytoplasm of apoptotic hepatocytes (black arrows). Cells with advanced loss of cellular detail characterized by indistinct cytoplasmic borders and complete nuclear fading, show indistinct fine diffuse immunolabelling (open arrows). Immunolabelling is difficult to detect or absent in fragmented cells (white arrowhead). Bar = 20 µm.

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Plate 5

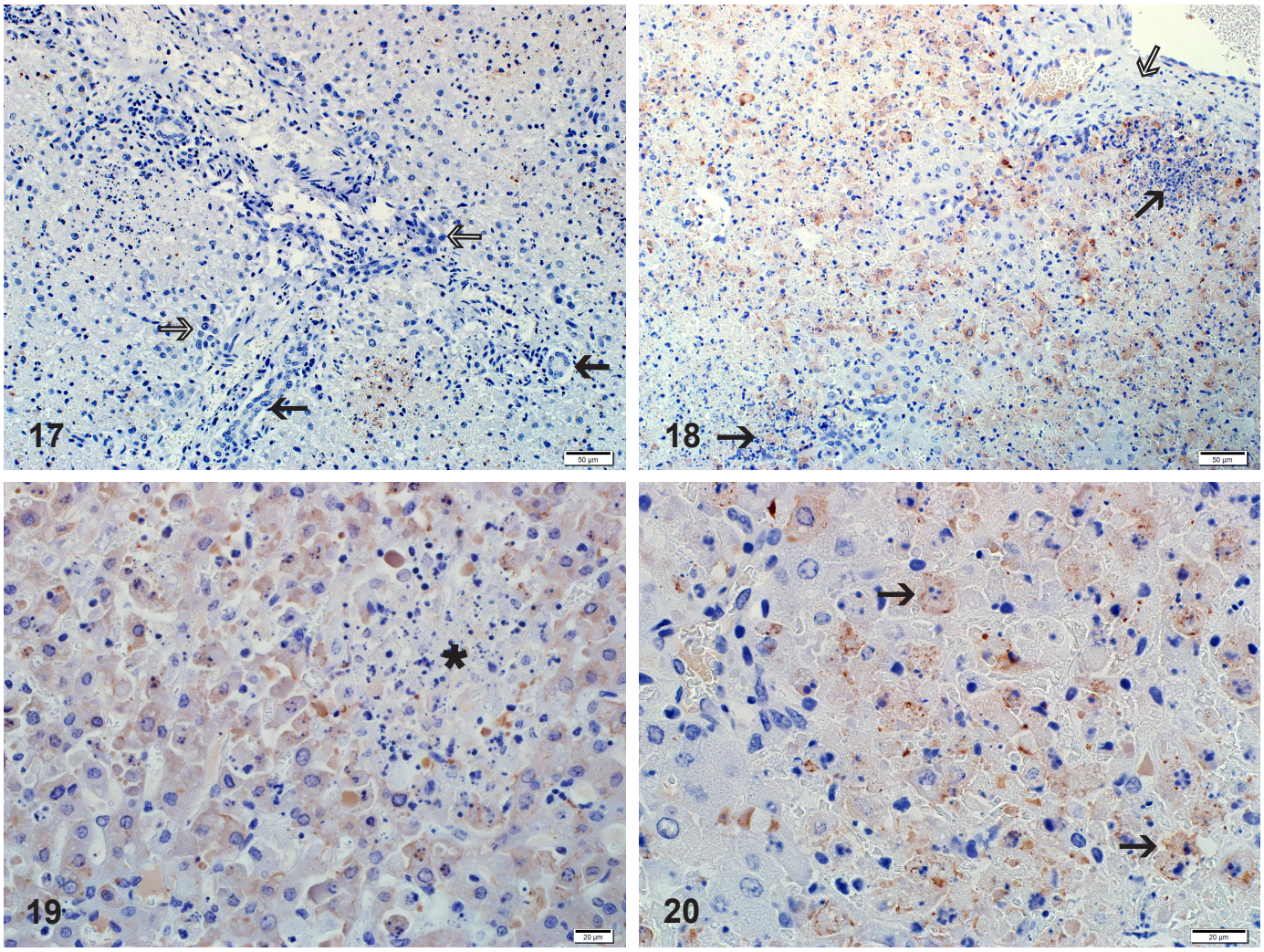


PLATE 5: Rift Valley fever virus antigen labelling in the liver of ovine and bovine neonates.

FIG.17: Bovine neonate (case 14). Positive immunolabelling is not observed within the portal tract bile duct epithelial cells (black arrows), or proliferating limiting plate epithelial cells (open arrows). Bar = 50 µm.

FIG.18: Ovine neonate (case 60). Positively immunolabeled cells, widely dispersed throughout the section indicate that virtually every hepatocyte contains Rift Valley fever viral antigen. Immunolabelling is largely absent from the primary foci of hepatocellular death (black arrows) and the connective tissue surrounding the portal artery (open arrow). Bar = 50 µm.

FIG.19: Ovine neonate (case 42). Immunolabelling is largely absent from the primary focus of hepatocellular death (asterisk). Bar = 20 µm.

FIG.20: Ovine neonate (case 60). Positive immunolabelling is prominent at the cytoplasmic margins of the injured hepatocytes (black arrows). Bar = 20 µm.

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Plate 6

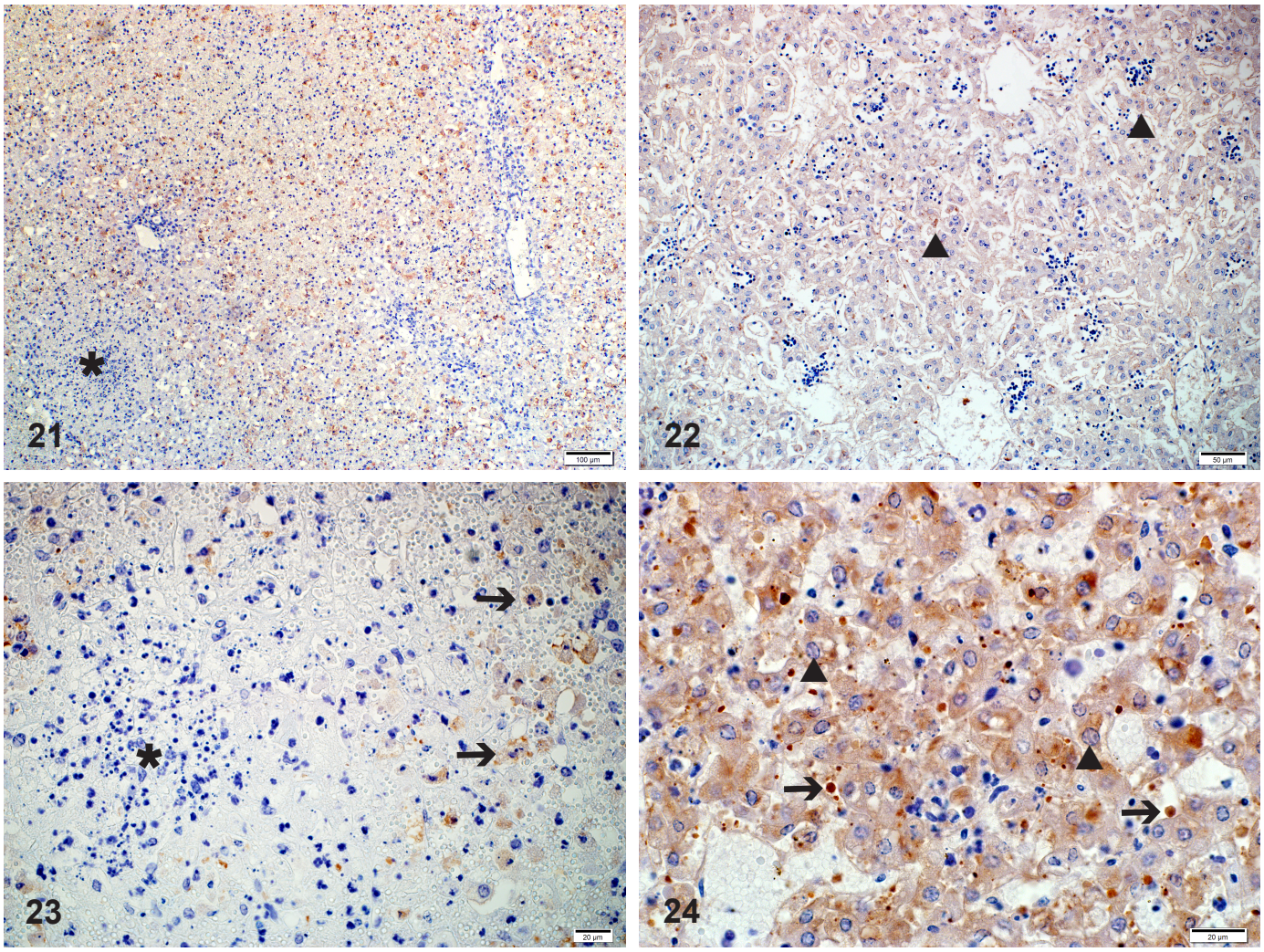


PLATE 6: Rift Valley fever virus antigen labelling in the liver of ovine foetuses.

FIG.21: Ovine foetus (case 39). Positively immunolabeled cells are widely dispersed throughout the section. Immunolabelling is only absent from the primary focus of hepatocellular death (asterisk). Bar = 100 µm.

FIG.22: Ovine foetus (case 199). Moderate extramedullary haematopoiesis in a young foetus with sparse convincing positive immunolabelling (black arrowheads). Bar = 50 µm.

FIG.23: Ovine foetus (case 39). Immunolabelling is not observed within the primary focus of hepatocellular death (asterisk). Hepatocytes at the edge of the primary focus show fine diffuse to coarse granular cytoplasmic immunolabelling (black arrows). Bar = 20 µm.

FIG.24: Ovine foetus (case 40). Fine diffuse to coarse granular cytoplasmic immunolabelling of apoptotic hepatocytes. Cytoplasmic fragments (black arrows) within the sinusoids are strongly labelled while immunolabelling is not associated with intranuclear inclusion bodies (black arrow heads). Bar = 20 µm.

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Plate 7

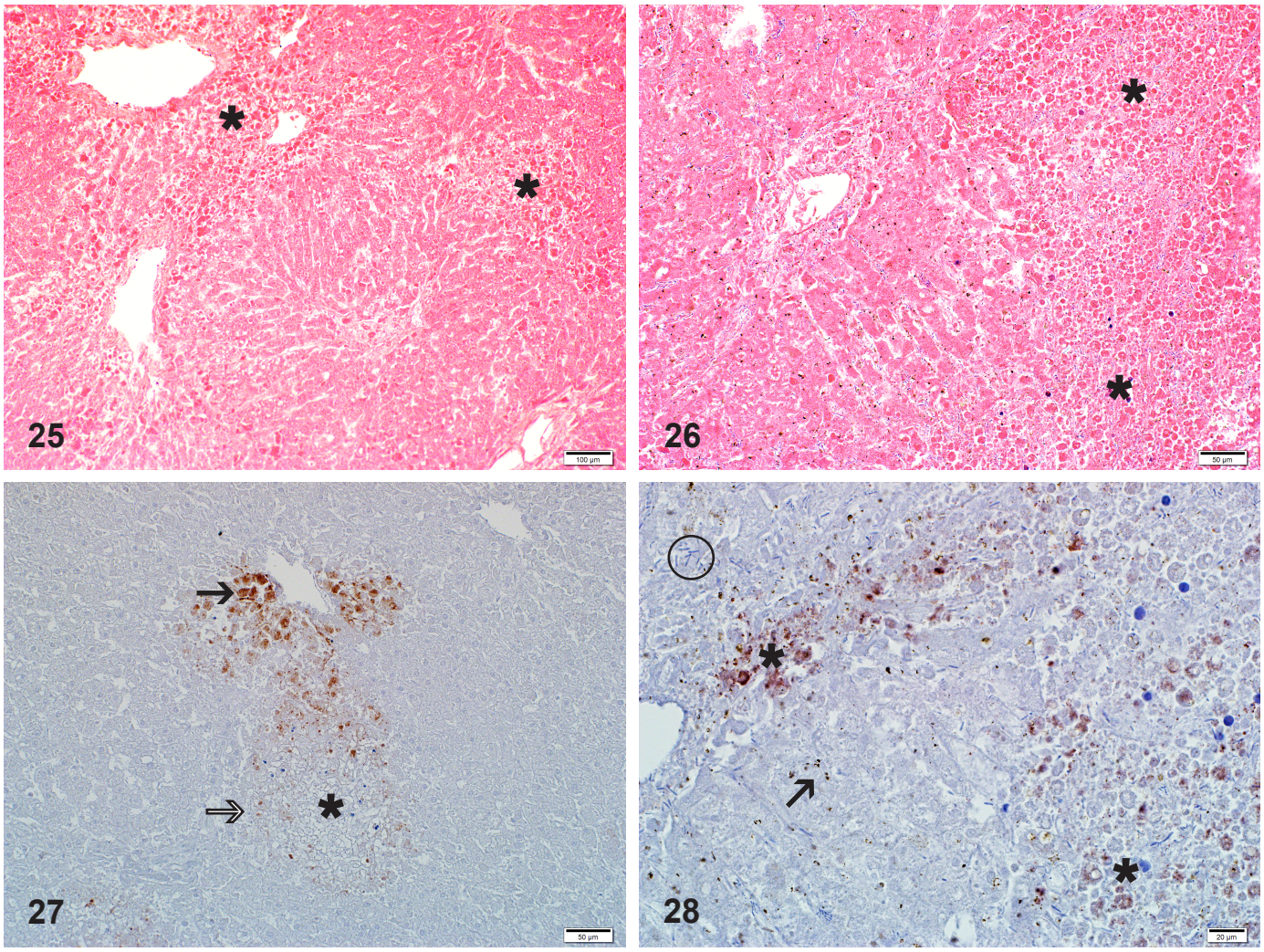


PLATE 7: Haematoxylin and eosin (HE)-staining, and Rift Valley fever virus antigen labelling, in autolysed liver specimens.

FIG. 25: Adult bovine (case 142). Multiple random foci of hepatocellular death (asterisks) can be discerned even in the presence of severe autolysis. Bar = 100 µm.

FIG. 26: Adult bovine (case 142). Rounded, disassociated cells, with hypereosinophilic cytoplasm (asterisks) in the periportal areas are suggestive of hepatocellular death due to apoptosis prior to severe autolysis. Bar = 50 µm.

FIG. 27: Adult bovine (case 63). Strong positive immunolabelling (black arrow) bordering the portal tract and involving cells within the limiting plate. Scattered positively labelled cells (open arrow) are present around an area reminiscent of a primary focus (asterisk). Bar = 50 µm.

FIG. 28: Adult bovine (case 142). Strong positive immunolabelling associated with apoptotic cells and cell fragments in the presence of severe autolysis (asterisks), putrefactive bacteria (circle), and acid haematin pigment (black arrow). Bar = 20 µm.

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Plate 8

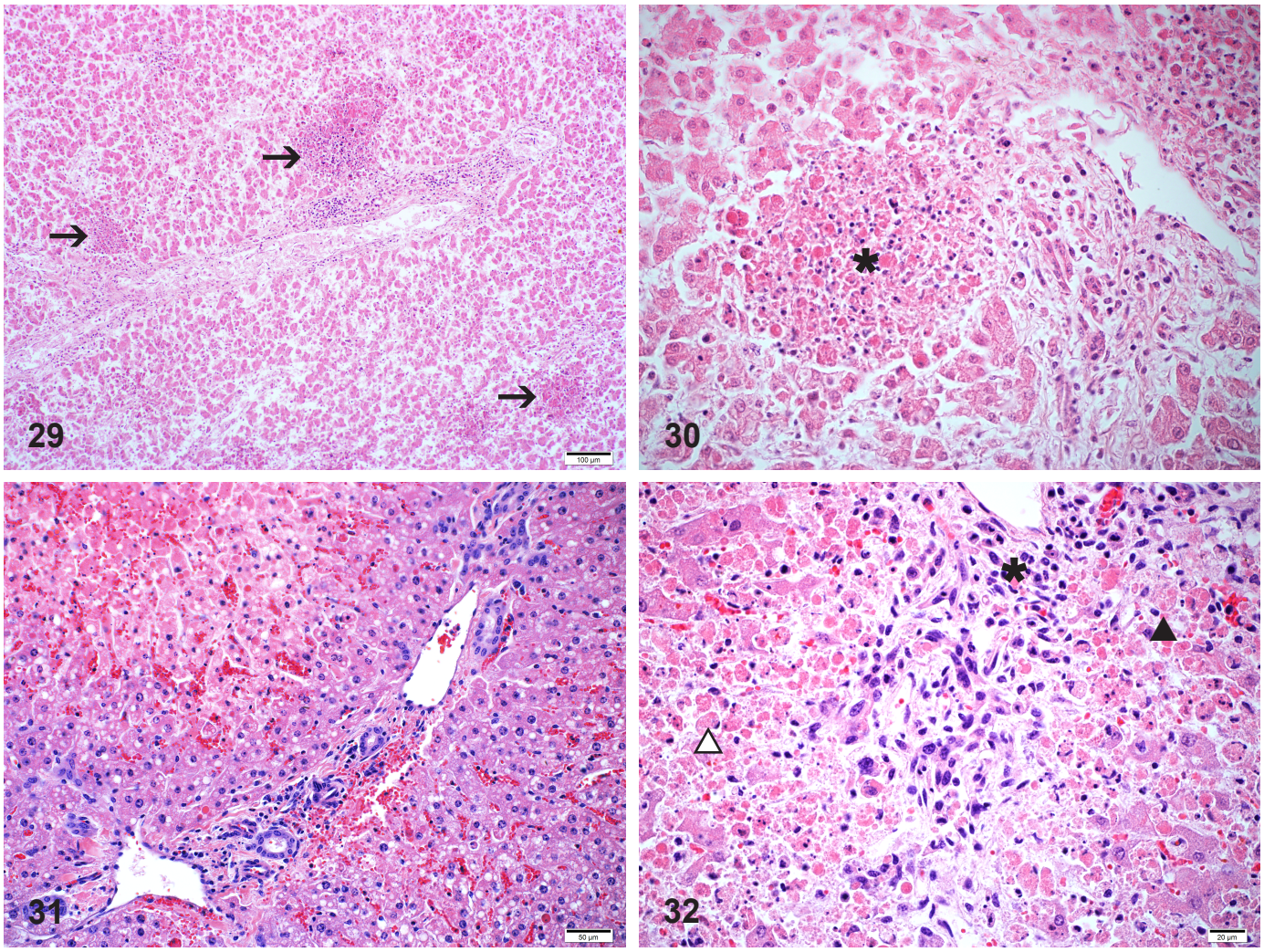


PLATE 8: Haematoxylin and eosin (HE)-stained sections of incorrectly classified cases.

FIG.29: Bovine foetus (case 258). Case of infectious bovine rhinotracheitis with multifocal random foci of hepatocellular death (black arrows), erroneously diagnosed as Rift Valley fever. Bar = 100 µm.

FIG.30: Bovine foetus (case 258). Focus of hepatocellular injury (asterisk) in a case of infectious bovine rhinotracheitis that superficially resembles primary foci described for early cases of Rift Valley fever infection. Bar = 20 µm.

FIG.31: Adult bovine (case 391). The researcher was blinded to the species and this case of Rift Valley fever in a bovine was erroneously diagnosed as Wesselsbron disease. Prominent periportal vacuolar degeneration and the absence of foci of hepatocellular death in the limiting plates were deemed inconsistent with lesions typical for Rift Valley fever infection. Bar = 50 µm.

FIG.32: Ovine neonate (case 423). Case of Wesselsbron disease diagnosed as Rift Valley fever. Lesions within the limiting plate, with rounded, disassociated, hypereosinophilic cells (white arrowhead) and fragmented cells (black arrowhead) accompanied by a mild infiltrate of mononuclear inflammatory cells in the portal tract (asterisk) was thought to be consistent with lesions typical for Rift Valley fever infection. Bar = 20 µm.

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but to a lesser degree. All these findings are in agreement with the analysis of the data set using the Bayesian latent class methods where diagnostic test accuracy was significantly lower in foetuses and severely autolysed specimens.

Inter-rater agreement for IHC was acceptable. Ninety eight percent (82/84) of the IHC results read by the two investigators were in agreement. Positive immunolabelling in two cases was missed due to reader error which is likely to increase when case loads are high or inexperienced personnel evaluate slides. One case was a foetus that had scattered positive immunolabelled cells. The second was a severely autolysed case where a few convincingly positive cells were detected by examining the slide under oil (100x magnification).

Table 7: Results for Cohen's kappa (unweighted) for histopathology measuring the level of agreement between two observers (reader 1 and reader 2) for the 84 cases where the test results for reverse transcription-polymerase chain reaction, histopathology and immunohistochemistry were not in perfect agreement (i.e. the results were not all positive or all negative).

	Histopathology	Immunohistochemistry
Sample population	0.32 (0.11 -0.53)	0.86 (0.65 - 1.07)
Age		
Adult	0.54 (0.27 - 0.82)	0.80 (0.52 - 1.09)
Neonate	0.38 (-0.05 - 0.80)	1.00 (0.55 - 1.45)
Foetus	-0.21 (-0.63 - 0.20)	0.85 (0.38 - 1.32)
Autolysis		
Mild	0.25 (-0.06 - 0.55)	0.87 (0.56 - 1.17)
Moderate	0.42 (-0.12 - 0.95)	0.83 (0.30 - 1.37)
Severe	0.35 (-0.01 - 0.70)	0.84 (0.49 - 1.19)
Pigments		
Absent	0.25 (-0.02 - 0.52)	0.91 (0.58 - 1.25)
Present	0.47 (0.16 - 0.78)	0.82 (0.54 - 1.10)

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Table 8: Results for Cohen's kappa (unweighted) for immunohistochemistry measuring the level of agreement between two observers (reader 1 and reader 2) where the calculations factored in those cases where the test results for reverse transcription-polymerase chain reaction, histopathology and immunohistochemistry were not in perfect agreement (i.e. the results were not all positive or all negative) as well as all the cases where all the test results obtained by the first reader was in agreement (i.e. all the results for all three tests positive or negative).

	Histopathology	Immunohistochemistry
Sample population	0.87 (0.77 - 0.97)	0.98 (0.88 - 1.08)
Age		
Adult	0.90 (0.77 - 1.03)	0.97 (0.83 - 1.11)
Neonate	0.90 (0.67 - 1.13)	1.00 (0.79 - 1.21)
Foetus	0.71 (0.47 - 0.95)	0.97 (0.77 - 1.18)
Autolysis		
Mild	0.90 (0.77 - 1.03)	0.98 (0.86 - 1.11)
Moderate	0.90 (0.67 - 1.13)	0.97 (0.74 - 1.20)
Severe	0.71 (0.47 - 0.95)	0.96 (0.72 - 1.21)
Pigments		
Absent	0.91 (0.76 - 1.06)	0.99 (0.83 - 1.14)
Present	0.83 (0.70 - 0.96)	0.97 (0.83 - 1.10)

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CHAPTER 5

DISCUSSION

A definitive diagnosis of RVF is challenging for a number of reasons. Primarily, the virus poses a health risk to laboratory personnel and routine diagnostic laboratories are precluded from performing diagnostic methods such as virus isolation, haemagglutination inhibition, polymerase chain reaction, or virus neutralization tests as prescribed by the World Animal Health Organization (OIE 2012, Gerdes 2004). Specimens that might contain infective virus particles should only be tested in biosecure reference laboratories, and preferably, by immunized personnel (Pepin *et al.* 2010). In addition, these tests often require expensive equipment, highly skilled laboratory technicians and continued support from companies that supply the materials and consumables required for the test (Pepin *et al.* 2010). A laboratory safe antigen detection ELISA employing a recombinant nucleocapsid protein has been developed (Pepin *et al.* 2010), and approved by the World Animal Health Organization. Low sensitivity (70%), however, suggests that this test is unsuitable for use during an epidemic. A further difficulty is that most of the recommended diagnostic techniques also require suitable fresh specimens to be collected and transported in a secure and contamination-free manner (Gerdes 2004). Suitable fresh specimens are often not available because the diagnosis was not suspected when the gross examination was conducted and formalin-fixed specimens are more conveniently dispatched over long distances.

In January 2010, an impending RVF outbreak in South Africa was suspected following exceptionally heavy rains, extremely high mortality rates among young lambs and gross and microscopic lesions typical of RVF. The diagnosis was confirmed using rRT-PCR and IHC and other diagnostic techniques were rarely used since they were either too lengthy to perform, too expensive or offered no advantage over the use of rRT-PCR and IHC. The present study attempted to estimate the diagnostic accuracy of histopathology, rRT-PCR and IHC using field samples and a Bayesian latent class approach.

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Under the conditions of this study, rRT-PCR was a highly sensitive assay for the detection and quantification of RVFV in liver specimens. The Se of rRT-PCR was 97.4% and only 6 cases (1.6%) tested negative for rRT-PCR but positive on both histopathology and IHC. However, the extraordinary analytical sensitivity of PCR makes this test very susceptible to false positive reactions (Burtis & Ashwood 2001) thus leading to an apparent reduced specificity. The Sp of rRT-PCR was 71.7% and 13.0% of cases (50/380) tested positive for rRT-PCR and negative on both histopathology and IHC. A cause for concern during large-scale disease outbreaks, where abortion storms are characteristic, is the risk for false positive reactions, due to viral contamination at sampling sites and equipment in the field, post-mortem halls and testing laboratories. Veterinary staff and the capacity of post-mortem examination facilities can be overwhelmed during an outbreak and many carcasses of animals that originate from different farms are frequently examined simultaneously in the same room. Another concern is that the procedures of sampling in the field and submission to the laboratory are often inappropriate, with excessive amounts of material being submitted for testing, often in inadequate containers that are not suitably leak-proof. Additionally, the processing of tissue specimens for nucleic acid extraction is tedious and prone to contamination risks. High levels of PCR products (amplicons) generated by large numbers of positive specimens can also contaminate other specimens, reagents, or work surfaces at the testing laboratory. This is particularly a problem because extremely high titres of virus can be present in tissue specimens (Pepin *et al.* 2010, McIntosh, Dickinson & Dos Santos 1973).

The Se (94.6%) and Sp (92.3%) of histopathology was higher than expected. Nine of the field cases (2.4%) were classified as positive on histopathology but tested negative on rRT-PCR and IHC. Single cases of RVF might be confused with acute poisoning with plants such as *Senecio* spp., *Crotalaria* spp., *Lasiospermum bipinnatum*, *Cestrum* spp., *Pteronia pallens* and *Hertia pallens*, and *Microcystis aeruginosa*, as well as bacterial septicaemias including pasteurellosis, salmonellosis and anthrax (Swanepoel & Coetzer 2004). Other

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abortifacient agents include *Brucella* spp., *Leptospira* spp, *Chlamydia* spp., *Campylobacter* spp. and *Coxiella burnetti* infection. Most of these conditions, however, can be excluded with histological examination of the liver, bacterial culture and toxicological or serological investigation (Swanepoel & Coetzer 2004). Two other more important differentials that might prove difficult to exclude using histopathology alone are WBD in sheep and IBR in bovine foetuses or neonates. Three of the 4 IBR cases and 1 of the 10 WBD cases were incorrectly classified as positive for RVF and 1 of the 2009 RVF cases was classified as WBD (Fig. 29, 30, 31, and 32). Microscopically, foci of hepatocellular injury in IBR cases may resemble the foci of cytolysis (primary foci) described for early cases of RVFV infection, and acute severe cases of WBD in sheep can resemble RVF.

Eleven of the field cases (2.9%) were classified as negative on histopathology but tested positive on rRT-PCR and IHC. A possible explanation is that early in the course of the disease (18 hours or less post infection) only isolated hepatocytes show evidence of injury, (Easterday *et al.* 1962b) and typical apoptosis and haemorrhage with a mild mixed inflammatory infiltrate is more readily identified as the disease progresses. The 11 cases were re-examined and hepatocellular injury with or without inflammation was detected in 8 cases but the pattern of cell injury or the type of inflammatory reaction was considered inconsistent with previous descriptions of the histopathology associated with RVFV. Other diseases such as *Escherichia coli* septicaemia, *Clostridial* spp. enteritis or mastitis, *Haemonchus contortus* paracitaemia or infection with a variety of arthropod-borne diseases such as bluetongue disease, *Coxiella burnetti* or *Anaplasma* spp. are not uncommon during the rainy season in South Africa. Co-infections with these disease agents, therefore, might explain why histopathology associated with RVFV was not recognized in some of the specimens. Sampling error might also be a contributing factor. Frequently only one section of liver is submitted for testing. However, RVFV affects the liver randomly and it is possible that a specimen might have been taken from a part of the liver where typical histological lesions had not yet developed or where liver injury had advanced to the point where antigen

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could no longer be detected using IHC. At least two cases were detected where two or more sections from the liver were examined revealing strong positive immunolabelling in one section with typical histological lesions but no lesions or positive immunolabelling in other sections.

The Se (97.6%) and Sp (99.4%) of IHC were quite high and none of the field cases were classified as positive for IHC but tested negative on rRT-PCR and histopathology. Additionally, all control cases were correctly classified. The high Sp of IHC is due to the fact that the characteristic positive immunolabelling of the cytoplasm of hepatocytes can be correlated with the presence of cytopathology typical for RVFV infection. Van der Lugt *et al.* (1996) studied the distribution of viral antigen in 12 new-born lambs infected with RVFV and detected positive immunolabelling in 11 of the lambs. Only small groups of injured hepatocytes labelled positive for RVFV in lambs euthanized early in the course of the disease whereas viral antigen was dispersed throughout the liver lobules in lambs euthanized later during the course of disease.

In contrast, 2% of the field cases (8/380) were classified positive on histopathology and rRT-PCR but tested negative on IHC. False negative results are sometimes obtained with IHC due to advanced autolysis and the loss of antigenic epitopes (Ramos-Vara 2005). The 8 cases were re-examined and 6 of them were found to be severely autolysed, which would support the presumption that antigenic epitopes become lost or too widely dispersed within cells due to disintegration of organelles or vesicles where viral proteins normally transit. False negative results are also reported to occur with prolonged fixation of specimens in formalin with a pH of 6 or less because those conditions cause the formation of acid haematin pigment (Ramos-Vara 2005). However, there was no convincing evidence that excessive acid haematin pigment caused false negative IHC results. Twenty-two percent of the cases (84/380) had substantial acid formalin and 52 of these cases were positive, and 24 negative, on all three tests. Only 9 had discordant test results and 7 of these were severely autolysed. Sampling error or reader error could also explain false negative IHC results.

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Viral proteins might be absent from completely destroyed sections of liver or scant positive immunolabelling can be missed, which is likely to increase when case loads are high or when inexperienced personnel evaluate slides.

The stratified analysis suggested differences in test accuracy in foetuses and severely autolysed specimens. The Sp of histopathology in foetuses (83.0%) was 9.3% lower than the value obtained for the sample population (92.3%). Five of the 9 field cases that were classified as positive on histopathology but tested negative on rRT-PCR and IHC, were foetuses. A clear-cut explanation as to why foetuses were more difficult to classify accurately could not be identified. No significant correlation could be detected between age of foetuses and imperfect test accuracy. In fact, very young foetuses (i.e. foetuses classified as showing severe EMH) were more accurately classified than older foetuses. Level of autolysis also does not seem to influence test accuracy in foetuses significantly since only 12% of the foetuses with inconsistent (2/17) test results showed severe autolysis. This emphasises the need to screen aborted foetuses in areas endemic for RVF using a variety of tests since a definitive diagnosis based on histopathology alone can be difficult when lesions are subtle.

In severely autolysed specimens, the Se of IHC decreased by 16.1% and the Sp of rRT-PCR by 17.4%. Twenty of the 65 cases (54%) classified as severely autolysed tested positive on all three tests while 15 tested negative on all three. Sixty-three percent (19/30) of the cases with discordant test results were negative on histopathology and IHC but positive on rRT-PCR. There is no plausible biological explanation for this decrease in the Sp of rRT-PCR. In fact, it would appear that RNA of RVFV is resistant to degradation in autolysing tissues. To mimic clearance of nucleocapsid protein in decomposing tissues from RVFV-infected animals, sheep and bovine RVFV spiked liver homogenate were incubated at 37 ^{O}C for a period of 8 days and tested at intervals with a sandwich ELISA developed for the detection of nucleocapsid protein of RVFV (Vuren & Paweska 2009). The ELISA detected antigen in spiked bovine and sheep liver homogenates up to at least 8 days. Conversely, the antibody

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used to detect RVFV using IHC detects epitopes raised against nucleoproteins of the virus and it is possible that viral proteins become too widely dispersed or degraded to detect by light microscopy. The assumption is that a marked decrease in Se of histopathology and IHC in severely autolysed specimens caused an apparent decrease in Sp of rRT-PCR. Therefore, IHC results from severely autolysed specimens should be interpreted with caution.

No attempt was made to estimate an unbiased population prevalence because the study was designed to estimate diagnostic accuracy. Therefore, no inferences can be drawn concerning the true prevalence of RVF during the 2010 outbreak in South Africa. However, the estimated prevalence obtained (52.7%) suggests that veterinarians or animal health technicians readily recognised the macroscopic lesions caused by RVFV and selected appropriate cases for testing. Seemingly, the typical lesions in sheep (56.8%) and neonates (56.9%) were more readily identified than in adult cattle (49.6%), which is in agreement with the findings of previous research (Coetzer & Ishak 1982, Coetzer 1977, Coetzer 1982, Daubney *et al.* 1931, Easterday *et al.* 1962a, Easterday *et al.* 1962b, Findlay 1932). Selection of cases from foetuses based on the occurrence of abortion storms had the highest prevalence (64.5%).

The repeatability analysis suggested histopathology of foetuses is more likely to lead to misclassified results. It was observed that many foetal cases had histological lesions that were typical for RVF such as apoptosis, nuclear inclusion bodies, or ellipsoid nuclei. However, some cases were very difficult to classify. Features such as a lack of a significant inflammatory response, nuclei with marginated chromatin but no inclusion bodies, cellular dropout that could not be accurately classified as necrosis or apoptosis as opposed to post mortal decomposition, often made it impossible to make an unequivocal decision. Conversely, adult animals, and neonates especially, can be correctly classified in most instances. Inter-rater agreement was also markedly reduced when specimens were severely autolysed making these likely to be misclassified. The repeatability with respect to

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cases where tissue pigments were present suggested that some of these cases might also be incorrectly classified. The presence of pigments such as bilirubin or haemosiderin can be associated with other differential diagnoses including *Lantana camara* poisoning or disease caused by haemoparasites. Qualitative assessment of cases, however suggested that this was not the case and it is not clear why inter-rater agreement decreased in this situation.

A limitation in this study was the restricted availability of suitable control specimens in foetuses. Additional cases of IBR infection in bovine foetuses would have been preferable to cases of EHV-1 infection in equine foetuses. However, EHV-1 cases were substituted since confirmed cases of IBR in foetuses is rare, and EHV-1 is the only disease causing hepatocellular injury in foetuses, for which an immunohistochemical test was developed at the faculty. It was further reasoned that foetal tissues of horses cannot be easily distinguished microscopically from those of cattle. The control cases that were used, however, served their intended purpose in this study, namely to compel the observer to motivate a decision of positive or negative for RVF in the case of histopathology or IHC circumspectly.

A further limitations was that severe autolysis in 17% of the cases (65/380) biased the Se and Sp result for histopathology and IHC. Likely, different results for Se and Sp will be obtained in different field situations (i.e. where all the samples are collected and submitted rapidly post mortally or much later). It also remains uncertain if the assumption that a marked decrease in Se of histopathology and IHC in severely autolysed specimens caused an apparent decrease in the Sp of rRT-PCR.

The hypothesis that co-infections with other disease agents might explain why histopathology associated with RVFV was not recognized in some of the specimens also needs to be investigated further.

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In conclusion, the high estimated Sp (99.4%) of IHC and the low Sp of rRT-PCR (71.3%) suggests that the definitive diagnosis or exclusion of RVF should not rely on a single PCR test and that IHC could be an effective confirmatory test for rRT-PCR positive field cases necropsied during an epidemic. The high Se of rRT-PCR (97.4%) and IHC (97.6%) suggest that both tests would be appropriate screening tests. It is also likely that the diagnostic Sp of rRT-PCR would increase substantially during periods when fewer mortalities due to RVFV infection occur and the risk of cross contamination of specimens is markedly reduced in post-mortem facilities or testing laboratories. Another advantage of rRT-PCR is that it can detect viraemia in blood samples of in contact animals and animals, such as adult cattle and goats, which are less likely to die during the course of infection. However, most countries have a limited number of biosecure reference laboratories that are able to perform rRT-PCR and the capacity of these facilities are rapidly overwhelmed during an outbreak. The diagnostic Se and Sp of histopathology was much higher than expected confirming the value of routine post mortem examinations and histopathology of liver specimens. Based on the availability of appropriate antibodies, the most feasible RVF testing option in countries that do not have suitable equipped laboratories, and where disease is often not diagnosed in livestock until after human cases, (Pepin *et al.* 2010) would be routine histopathology screening with IHC confirmation. In this respect, an important advantage of IHC is that it reduces the human health risk during shipment because it is based on formalin-fixed specimens that will not contain infectious virus and therefore can be safely transported over long distances or even across international borders.

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APPENDIXES

APPENDIX 1: Database of all Rift Valley fever test cases included in the study.

Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
493-12	1	411	Ovine	Adult	Positive	Negative	Positive	Severe	None	Mild
492-12	2	17	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	Mild
824-12 A	3	211	Ovine	Adult	Negative	Negative	Negative	Moderate	None	Mild
975-10	4	447	Bovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
2170-10	5	374	Ovine	Adult	Positive	Positive	Positive	Mild	None	Mild
1890-12	6	327	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1693-12	7	345	Ovine	Adult	Positive	Negative	Negative	Mild	None	None
1690-12	8	241	Ovine	Foetus	Positive	Negative	Negative	Mild	None	None
1622-12	9	14	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
1705-12	10	305	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
478-12	11	352	Bovine	Neonate	Negative	Negative	Negative	Severe	None	None
536-12	13	101	Ovine	Neonate	Positive	Positive	Positive	Mild	Severe	None
857-10	14	124	Bovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
553-12	15	324	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
481-12	16	73	Ovine	Adult	Positive	Negative	Negative	Severe	Moderate	None
1599-12	17	296	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1650-12	18	425	Bovine	Foetus	Positive	Negative	Negative	Mild	None	None
1574-12 B	19	326	Bovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
552-12	20	401	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1708-12	22	94	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
494-12 C	23	35	Ovine	Foetus	Positive	Negative	Negative	Mild	Mild	None
643-10	24	275	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
538-12	26	399	Bovine	Foetus	Negative	Negative	Negative	Severe	None	None
565-12 A	27	280	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	None
477-12	28	342	Bovine	Neonate	Positive	Negative	Negative	Mild	Mild	Mild
1613-12	29	144	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	Mild
1606-12	30	370	Caprine	Adult	Negative	Negative	Negative	Mild	None	Mild
812-12	31	329	Bovine	Adult	Positive	Negative	Negative	Mild	None	Mild
490-12	32	216	Bovine	Adult	Positive	Positive	Positive	Severe	Severe	None
1646-12	33	299	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
1666-10	34	206	Bovine	Foetus	Positive	Positive	Positive	Moderate	None	None
1608-12	35	438	Ovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
1644-12	36	153	Ovine	Neonate	Negative	Negative	Negative	Mild	None	Mild
494-12 B	37	343	Ovine	Foetus	Positive	Positive	Positive	Moderate	Severe	Mild
577-12	38	371	Caprine	Adult	Negative	Negative	Negative	Mild	None	Mild
1210-10	39	164	Ovine	Foetus	Positive	Positive	Positive	Mild	Moderate	None
942-10 C	40	380	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
616-12 B	42	64	Ovine	Neonate	Negative	Positive	Positive	Mild	None	None
597-12 A	43	298	Ovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
1574-12 A	44	15	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
561-12	45	386	Ovine	Adult	Negative	Negative	Negative	Moderate	None	None
532-12	46	242	Ovine	Foetus	Positive	Positive	Positive	Moderate	Moderate	None
585-12 A	47	93	Ovine	Adult	Positive	Positive	Negative	Moderate	Mild	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1701-12	48	459	Bovine	Foetus	Positive	Positive	Positive	Moderate	Severe	None
589-10	49	210	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1623-12	51	26	Ovine	Neonate	Positive	Positive	Positive	Moderate	None	Mild
1627-12	52	290	Ovine	Neonate	Negative	Positive	Positive	Mild	Severe	None
1410-10	53	74	Ovine	Adult	Negative	Negative	Positive	Mild	Severe	Mild
1324-10	54	337	Bovine	Foetus	Negative	Negative	Negative	Moderate	Mild	None
1549-10	55	226	Bovine	Foetus	Positive	Negative	Negative	Mild	None	Mild
486-12	56	172	Bovine	Adult	Positive	Positive	Positive	Mild	None	None
1619-12	57	225	Ovine	Adult	Positive	Positive	Positive	Moderate	Severe	Mild
592-12 C	58	431	Ovine	Adult	Negative	Negative	Negative	Mild	None	Mild
1699-12	59	268	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1350-10 A	60	100	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
814-12 B	62	176	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
1596-12	63	448	Bovine	Adult	Positive	Positive	Positive	Severe	Mild	None
595-12	64	165	Ovine	Adult	Positive	Negative	Negative	Severe	None	None
1666-12	65	52	Ovine	Neonate	Positive	Positive	Positive	Mild	Mild	Mild
1680-12 C	66	437	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
1588-12	67	259	Bovine	Adult	Positive	Positive	Positive	Mild	None	Moderate
544-12	68	304	Bovine	Adult	Positive	Positive	Positive	Mild	None	Mild
1684-12	69	41	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
471-12	70	436	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1640-12	71	40	Ovine	Foetus	Positive	Positive	Positive	Mild	None	Mild
593-12 A	72	351	Ovine	Neonate	Positive	Negative	Negative	Severe	None	None
810-12	73	8	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
2005-10	74	416	Ovine	Adult	Negative	Negative	Negative	Mild	None	Severe
1011-10	75	123	Ovine	Adult	Negative	Negative	Negative	Moderate	None	None
548-12	76	362	Bovine	Adult	Negative	Negative	Negative	Moderate	Mild	None
537-12	77	157	Ovine	Adult	Positive	Positive	Positive	Mild	None	Mild
468-12	78	269	Ovine	Foetus	Positive	Positive	Positive	Moderate	Severe	None
488-12	79	289	Ovine	Adult	Positive	Negative	Positive	Severe	None	Mild
1888-12	80	87	Ovine	Neonate	Positive	Negative	Negative	Severe	Mild	None
845-12 A	81	201	Ovine	Foetus	Negative	Negative	Negative	Mild	Mild	None
1894-12	83	125	Ovine	Adult	Negative	Negative	Negative	Severe	None	None
937-10	84	47	Ovine	Adult	Positive	Positive	Positive	Moderate	None	None
1582-12	85	183	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
500-12 B	86	382	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
608-12	87	12	Ovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
1634-12	88	169	Ovine	Foetus	Negative	Negative	Negative	Mild	Severe	None
793-10	89	31	Bovine	Adult	Positive	Negative	Negative	Mild	None	None
542-12	90	212	Ovine	Adult	Positive	Positive	Positive	Mild	Severe	None
1698-12	91	145	Caprine	Adult	Negative	Negative	Negative	Mild	None	None
594-12	92	49	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
1659-12	93	230	Ovine	Neonate	Negative	Negative	Negative	Mild	Mild	None
529-12	94	213	Bovine	Adult	Positive	Positive	Positive	Severe	None	None
551-12	95	173	Ovine	Neonate	Negative	Negative	Negative	Moderate	None	None
1443-10	96	262	Bovine	Adult	Positive	Negative	Negative	Mild	None	Mild
580-12	97	407	Bovine	Adult	Positive	Positive	Positive	Moderate	Mild	Mild
603-12	98	265	Bovine	Neonate	Positive	Negative	Negative	Mild	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
475-12 †	99	166	Ovine	Adult	Positive	-	-	-	-	-
518-12	100	207	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1680-12 A	101	334	Ovine	Neonate	Negative	Negative	Negative	Mild	None	Severe
845-12 B	102	288	Ovine	Adult	Negative	Negative	Negative	Mild	None	Mild
592-12 A	103	141	Ovine	Neonate	Negative	Negative	Negative	Moderate	Severe	None
609-12	104	114	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1672-12	105	136	Ovine	Foetus	Positive	Positive	Positive	Moderate	Mild	None
514-12	106	199	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	None
619-12	107	354	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
811-12 †	108	361	Bovine	Adult	Positive	-	-	-	-	-
1645-12	110	281	Ovine	Neonate	Positive	Positive	Positive	Mild	Mild	Mild
1615-12	111	209	Bovine	Adult	Positive	Positive	Positive	Moderate	Moderate	Mild
491-12	112	161	Bovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
603-10 A	113	158	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1671-12	114	196	Bovine	Adult	Negative	Negative	Negative	Moderate	None	Mild
1628-12	115	278	Ovine	Foetus	Negative	Negative	Negative	Mild	None	None
579-12	116	395	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	None
1668-12	117	408	Ovine	Adult	Positive	Negative	Negative	Severe	Moderate	None
568-12	118	20	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1581-12	119	332	Bovine	Adult	Positive	Positive	Positive	Severe	None	Mild
578-12	120	323	Ovine	Adult	Negative	Negative	Negative	Mild	Moderate	Mild
563-10	121	11	Bovine	Adult	Positive	Positive	Positive	Mild	None	Mild
494-12 A	122	355	Ovine	Foetus	Positive	Positive	Positive	Moderate	Moderate	None
1684-10	123	81	Bovine	Adult	Negative	Negative	Negative	Mild	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1680-12 B †	124	24	Ovine	Neonate	Negative	-	-	-	-	-
1649-12	125	256	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
587-12	126	333	Ovine	Adult	Positive	Positive	Positive	Mild	None	Severe
509-12 A	127	317	Ovine	Adult	Positive	Positive	Positive	Severe	None	None
1592-12	128	308	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
549-12	129	384	Bovine	Adult	Positive	Positive	Positive	Severe	Mild	None
603-10 B	130	291	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1586-12	131	426	Bovine	Adult	Positive	Positive	Positive	Mild	None	Mild
813-12	132	445	Bovine	Adult	Positive	Negative	Negative	Severe	Mild	None
547-12	133	339	Bovine	Adult	Positive	Positive	Positive	Severe	None	None
1696-12	134	251	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
1584-12	135	132	Bovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
572-12	136	181	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
469-12 A	137	237	Ovine	Foetus	Positive	Positive	Positive	Mild	Moderate	None
1030-10 A	138	36	Ovine	Adult	Positive	Positive	Positive	Mild	None	Mild
1673-12	139	71	Bovine	Adult	Positive	Negative	Negative	Mild	None	Moderate
1059-10	142	452	Bovine	Adult	Positive	Positive	Positive	Severe	Moderate	None
593-12 B	143	248	Ovine	Neonate	Positive	Negative	Negative	Severe	None	None
1106-10	144	458	Bovine	Foetus	Negative	Negative	Negative	Moderate	Severe	None
605-12	146	270	Ovine	Adult	Negative	Negative	Negative	Severe	None	Mild
942-10 B †	147	417	Ovine	Adult	Negative	-	-	-	-	-
615-12	148	306	Caprine	Adult	Negative	Negative	Negative	Mild	None	Mild
1704-12	149	177	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
500-12 A	150	320	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Mild

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
516-10	151	240	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	Mild
1638-12 †	152	190	Bovine	Adult	Positive	-	-	-	-	-
1665-12 B	153	346	Ovine	Neonate	Positive	Positive	Negative	Mild	None	None
622-12	154	99	Bovine	Neonate	Negative	Negative	Negative	Mild	None	None
560-12	155	422	Caprine	Adult	Positive	Positive	Positive	Mild	Mild	Mild
1639-12	156	253	Ovine	Foetus	Negative	Negative	Negative	Mild	None	None
573-12	158	82	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
1632-12	159	433	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
586-12	160	180	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	None
819-10	161	194	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
485-12	162	27	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
846-12	163	186	Ovine	Adult	Positive	Negative	Positive	Severe	Severe	None
582-12	164	191	Ovine	Foetus	Positive	Positive	Negative	Severe	Mild	None
809-12 A	165	266	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
1677-12	166	171	Caprine	Foetus	Negative	Negative	Negative	Mild	None	Mild
1887-12	167	284	Ovine	Neonate	Positive	Positive	Positive	Moderate	None	None
563-12	168	314	Ovine	Adult	Positive	Positive	Negative	Mild	None	Mild
2157-10 A	169	258	Ovine	Adult	Positive	Positive	Positive	Moderate	None	Mild
818-12	170	369	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1198-10 A	171	264	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	Moderate
1674-12	173	108	Caprine	Foetus	Negative	Negative	Negative	Mild	None	Severe
1889-12	174	214	Ovine	Adult	Positive	Negative	Positive	Severe	None	None
602-12	175	59	Ovine	Neonate	Positive	Negative	Negative	Moderate	None	None
1578-12	176	104	Bovine	Adult	Positive	Positive	Positive	Moderate	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
487-12 C	177	389	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
505-12	178	134	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None
1603-12	179	373	Ovine	Adult	Positive	Positive	Positive	Severe	Moderate	None
1577-12	180	61	Bovine	Adult	Positive	Positive	Positive	Mild	None	None
520-12	181	95	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
1662-12 †	182	192	Ovine	Neonate	Negative	-	-	-	-	-
1686-12 A †	183	111	Bovine	Foetus	Negative	-	-	-	-	-
1700-12	184	372	Caprine	Adult	Negative	Negative	Negative	Mild	None	Mild
1892-12	185	79	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
616-12 A	186	367	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1702-12	187	420	Ovine	Foetus	Negative	Negative	Negative	Moderate	None	Mild
1593-12 B	188	121	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
499-12	189	203	Ovine	Adult	Negative	Negative	Negative	Severe	Moderate	None
1629-12	190	28	Bovine	Adult	Negative	Negative	Positive	Severe	None	Mild
1686-12 B	191	150	Bovine	Foetus	Negative	Negative	Negative	Mild	None	None
1636-12	192	118	Caprine	Adult	Positive	Positive	Positive	Moderate	Mild	None
1718-10	193	391	Ovine	Adult	Negative	Negative	Negative	Severe	Moderate	Mild
2366-10	194	156	Bovine	Foetus	Positive	Positive	Negative	Mild	None	Moderate
1663-12	195	441	Caprine	Neonate	Negative	Negative	Negative	Mild	Mild	None
617-12	196	188	Ovine	Foetus	Negative	Negative	Negative	Moderate	None	None
484-12 A	198	286	Ovine	Neonate	Positive	Negative	Negative	Severe	None	None
598-12	199	89	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
814-12 A	200	239	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
1232-10	201	283	Bovine	Neonate	Positive	Positive	Positive	Moderate	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1198-10 B	202	129	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	Moderate
1591-12	203	1	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
1618-12	204	80	Ovine	Neonate	Positive	Positive	Positive	Severe	Mild	None
2171-10 A	205	184	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
515-12	207	38	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None
1580-12 A	208	83	Ovine	Foetus	Positive	Negative	Negative	Moderate	None	None
1648-12	209	22	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
1329-10 A	210	149	Bovine	Neonate	Positive	Negative	Negative	Mild	None	None
522-12 †	211	366	Bovine	Foetus	Positive	-	-	-	-	-
1697-12 B	212	91	Ovine	Neonate	Negative	Negative	Negative	Mild	None	Mild
1685-12 B	213	409	Ovine	Foetus	Negative	Negative	Negative	Severe	Mild	None
1594-12 A	214	233	Ovine	Foetus	Positive	Negative	Negative	Mild	None	Mild
1602-12	215	62	Ovine	Adult	Positive	Positive	Positive	Moderate	Mild	None
506-12	216	110	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1350-10 B	217	412	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1626-12	218	312	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
1691-12	219	375	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
1641-12	220	137	Ovine	Adult	Positive	Negative	Negative	Severe	Mild	None
1937-10	221	217	Caprine	Adult	Negative	Negative	Negative	Mild	None	None
508-12	223	331	Ovine	Foetus	Positive	Positive	Negative	Moderate	None	None
613-12	224	414	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
498-12 †	225	249	Bovine	Foetus	Negative	-	-	-	-	-
583-12	226	440	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
1073-10 A	227	316	Bovine	Foetus	Negative	Negative	Negative	Mild	Severe	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
511-12	228	223	Bovine	Adult	Positive	Negative	Negative	Moderate	None	None
1695-12	229	446	Ovine	Neonate	Positive	Negative	Negative	Mild	Moderate	Moderate
606-12	230	350	Ovine	Foetus	Positive	Positive	Positive	Mild	Severe	None
1616-12	231	338	Bovine	Adult	Positive	Negative	Negative	Severe	None	None
1590-12	232	231	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
571-12	233	303	Bovine	Adult	Negative	Negative	Negative	Severe	Moderate	None
817-10	234	236	Ovine	Foetus	Negative	Negative	Negative	Moderate	None	None
1617-12	235	319	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
621-12	236	109	Bovine	Adult	Positive	Positive	Positive	Mild	Mild	None
1678-12	237	122	Bovine	Foetus	Negative	Negative	Negative	Mild	None	None
484-12 B	238	307	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	Mild
476-12	239	105	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
562-10	240	53	Bovine	Adult	Positive	Positive	Positive	Mild	Mild	None
545-12	241	170	Ovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
526-12	242	293	Ovine	Foetus	Negative	Negative	Negative	Mild	None	None
484-12 C	244	315	Ovine	Adult	Positive	Positive	Positive	Severe	None	None
1605-12	245	335	Ovine	Neonate	Positive	Positive	Negative	Mild	None	None
819-12	246	168	Ovine	Adult	Positive	Negative	Negative	Severe	None	None
1349-10	248	328	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
479-12	249	227	Bovine	Adult	Negative	Positive	Positive	Severe	None	None
1325-10	250	115	Bovine	Foetus	Positive	Positive	Positive	Moderate	Mild	Mild
1600-12	251	394	Ovine	Foetus	Negative	Negative	Negative	Mild	None	Mild
527-12	252	202	Ovine	Adult	Negative	Negative	Negative	Severe	Moderate	None
618-12	255	187	Ovine	Neonate	Positive	Negative	Negative	Severe	Mild	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1575-12	256	159	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
543-12	260	295	Ovine	Adult	Positive	Negative	Negative	Mild	None	Mild
1601-12	261	45	Ovine	Foetus	Negative	Negative	Positive	Mild	None	None
1031-10	262	451	Ovine	Adult	Positive	Positive	Negative	Mild	None	None
507-12	263	5	Ovine	Foetus	Positive	Positive	Positive	Mild	None	Mild
915-10	264	25	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	Moderate
1742-10	265	309	Ovine	Adult	Positive	Negative	Negative	Severe	Mild	None
1738-10	266	341	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
917-10	267	4	Bovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
1712-10	268	151	Ovine	Adult	Negative	Negative	Negative	Mild	Severe	None
1048-10	269	7	Ovine	Foetus	Negative	Negative	Negative	Mild	Severe	None
1576-12 A	270	182	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
509-12 B	271	460	Ovine	Adult	Positive	Negative	Negative	Severe	Mild	None
823-12	272	271	Ovine	Neonate	Negative	Negative	Negative	Severe	None	None
483-12	273	313	Ovine	Adult	Positive	Negative	Negative	Severe	None	None
1647-12	274	300	Ovine	Adult	Positive	Negative	Negative	Mild	Mild	Mild
1655-12	275	56	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
985-10	276	66	Bovine	Adult	Positive	Positive	Positive	Moderate	Severe	None
815-12	277	117	Ovine	Foetus	Positive	Negative	Positive	Severe	None	Mild
1182-10	278	195	Bovine	Adult	Positive	Positive	Positive	Mild	Severe	Mild
996-10	279	282	Ovine	Foetus	Negative	Negative	Negative	Severe	Severe	None
504-12	280	63	Ovine	Adult	Positive	Positive	Positive	Mild	None	Mild
590-12	281	235	Bovine	Foetus	Negative	Negative	Negative	Moderate	None	None
614-12 †	282	43	Ovine	Adult	Positive	-	-	-	-	-

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
470-12	283	455	Ovine	Neonate	Positive	Positive	Positive	Moderate	Moderate	None
1049-10 B	284	107	Bovine	Adult	Negative	Negative	Positive	Mild	None	None
581-12	285	310	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
1679-12	286	456	Caprine	Foetus	Negative	Negative	Negative	Mild	None	Mild
540-12	288	428	Bovine	Adult	Positive	Positive	Positive	Mild	None	Mild
1633-12	289	138	Ovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
664-10	290	131	Bovine	Adult	Positive	Positive	Positive	Mild	None	None
1585-12	291	84	Bovine	Adult	Positive	Positive	Positive	Moderate	Moderate	None
1593-12 A †	292	54	Bovine	Neonate	Negative	-	-	-	-	-
1692-12	293	51	Bovine	Adult	Negative	Negative	Negative	Moderate	None	Mild
531-12	294	439	Ovine	Adult	Negative	Positive	Negative	Mild	Severe	None
1609-12 A	295	292	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1614-12	296	450	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	None
516-12	297	140	Ovine	Adult	Positive	Positive	Positive	Severe	None	None
567-12	298	435	Ovine	Adult	Positive	Positive	Negative	Severe	None	None
530-12	299	397	Ovine	Foetus	Negative	Negative	Positive	Mild	Severe	None
1711-12	300	364	Ovine	Neonate	Positive	Negative	Negative	Mild	None	None
1329-10 B	301	120	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None
1612-12	302	273	Ovine	Neonate	Negative	Negative	Negative	Moderate	None	Moderate
566-12	303	39	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
554-12	304	90	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
1697-12 A	305	424	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
1029-10	306	106	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None
1286-10	307	174	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1620-12	308	135	Ovine	Adult	Negative	Negative	Negative	Mild	Moderate	None
1689-12 †	309	430	Ovine	Foetus	Negative	-	-	-	-	-
569-12	310	205	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
553-12 B	311	204	Ovine	Adult	Negative	Negative	Negative	Mild	Mild	None
1630-12	313	197	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1624-12	314	142	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	None
1607-12	315	179	Ovine	Foetus	Positive	Positive	Positive	Severe	Mild	None
565-12 B	316	126	Ovine	Neonate	Positive	Negative	Negative	Moderate	Mild	None
1533-10 B	317	29	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1625-12	318	443	Ovine	Foetus	Positive	Positive	Positive	Moderate	Mild	None
1631-12	319	19	Bovine	Adult	Negative	Negative	Negative	Moderate	Mild	None
575-12	320	112	Caprine	Foetus	Negative	Negative	Negative	Mild	Moderate	None
841-12	321	360	Ovine	Adult	Negative	Negative	Negative	Severe	Mild	None
1680-12 D	322	13	Ovine	Neonate	Negative	Negative	Negative	Mild	None	Mild
576-12	323	42	Ovine	Neonate	Negative	Negative	Positive	Severe	Moderate	None
539-12 †	324	461	Ovine	Adult	Positive	-	-	-	-	-
628-10	325	402	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1637-12	326	6	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
1597-12	327	294	Ovine	Neonate	Negative	Negative	Negative	Severe	Severe	None
574-12	328	356	Bovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
1583-12	329	33	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
1709-12	330	208	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
847-12	331	72	Bovine	Adult	Negative	Negative	Negative	Moderate	None	None
564-12	333	229	Ovine	Adult	Negative	Negative	Negative	Mild	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1680-12 E	334	154	Ovine	Neonate	Negative	Negative	Negative	Mild	None	Mild
1664-12	336	387	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
610-12	337	272	Ovine	Foetus	Positive	Positive	Positive	Moderate	Mild	None
510-12	338	254	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
2171-10 B	340	421	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1604-12 A	341	358	Caprine	Neonate	Negative	Negative	Negative	Mild	Severe	None
487-12 A †	342	18	Ovine	Adult	Positive	-	-	-	-	-
1609-12 B	343	381	Caprine	Foetus	Positive	Positive	Positive	Moderate	Severe	None
817-12	344	97	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
1694-12	345	103	Bovine	Adult	Positive	Negative	Negative	Severe	Severe	None
1682-12	346	116	Caprine	Adult	Negative	Negative	Negative	Moderate	None	Mild
1893-12	347	247	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
519-12	348	261	Bovine	Neonate	Positive	Negative	Negative	Mild	None	None
604-12	349	427	Ovine	Adult	Negative	Negative	Negative	Severe	None	Mild
1328-10	350	23	Bovine	Foetus	Negative	Negative	Positive	Moderate	None	Mild
489-12 †	351	9	Bovine	Adult	Positive	-	-	-	-	-
943-10	352	454	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
620-12	353	244	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
821-12	354	257	Bovine	Neonate	Positive	Negative	Negative	Mild	None	None
495-12	355	189	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
591-12	356	139	Bovine	Adult	Negative	Negative	Negative	Mild	None	None
1665-12 A	357	21	Ovine	Adult	Positive	Positive	Negative	Mild	None	Moderate
503-12	359	127	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
533-12	360	347	Bovine	Adult	Positive	Positive	Negative	Moderate	Severe	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1598-12	361	410	Ovine	Adult	Positive	Positive	Positive	Severe	Severe	None
496-12	362	444	Ovine	Foetus	Positive	Positive	Positive	Mild	Severe	Mild
480-12	363	37	Ovine	Foetus	Positive	Positive	Positive	Mild	None	None
553-12 C	364	50	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1652-12	365	287	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
942-10 A †	366	330	Ovine	Adult	Negative	-	-	-	-	-
1593-12 C	367	102	Bovine	Foetus	Positive	Positive	Positive	Moderate	None	Mild
1580-12 B	368	390	Ovine	Foetus	Negative	Positive	Positive	Moderate	None	Mild
1670-12 B	369	10	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
625-12	371	222	Ovine	Adult	Positive	Negative	Negative	Mild	None	None
501-12	372	234	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
467-12	373	336	Bovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
1533-10 A	374	3	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
1040-10	375	2	Bovine	Adult	Negative	Positive	Positive	Moderate	Moderate	None
1658-12	376	413	Bovine	Adult	Positive	Negative	Negative	Mild	None	None
523-12	377	302	Bovine	Foetus	Positive	Positive	Positive	Mild	None	Mild
600-12	378	340	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
824-12 B	379	415	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1860-10	381	325	Ovine	Neonate	Positive	Positive	Positive	Moderate	None	None
820-12	382	423	Ovine	Foetus	Positive	Positive	Positive	Severe	Severe	None
487-12 B	383	75	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
562-12	384	219	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1587-12	385	155	Bovine	Neonate	Positive	Positive	Positive	Mild	None	None
616-12 C †	386	457	Ovine	Adult	Negative	-	-	-	-	-

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
534-12	387	69	Ovine	Adult	Positive	Positive	Positive	Severe	Moderate	None
1685-12 A †	388	398	Ovine	Foetus	Negative	-	-	-	-	-
1667-12	389	218	Ovine	Adult	Positive	Negative	Negative	Mild	None	Mild
1595-12	390	344	Bovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
570-12	392	383	Ovine	Adult	Positive	Negative	Negative	Moderate	None	Mild
473-12	393	318	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
596-12	394	163	Ovine	Adult	Positive	Negative	Negative	Mild	None	Mild
1604-12 B	395	85	Caprine	Adult	Negative	Negative	Negative	Severe	None	Moderate
1707-12	397	385	Bovine	Adult	Positive	Positive	Positive	Mild	None	None
1675-12	398	311	Bovine	Neonate	Negative	Negative	Negative	Mild	Severe	None
1642-12	399	77	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
1643-12	400	377	Ovine	Adult	Positive	Positive	Positive	Severe	None	None
2168-10	401	96	Bovine	Adult	Positive	Positive	Positive	Mild	None	None
1621-12	402	119	Ovine	Adult	Positive	Positive	Positive	Mild	Moderate	None
1688-12	403	267	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
524-12	404	55	Bovine	Adult	Positive	Negative	Negative	Severe	Severe	None
1635-12	405	255	Ovine	Adult	Negative	Negative	Negative	Mild	Severe	None
1681-12	406	67	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
816-12 †	407	57	Ovine	Foetus	Negative	-	-	-	-	-
2158-10	408	263	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	Moderate
555-12	409	434	Bovine	Foetus	Positive	Positive	Positive	Mild	None	None
1073-10 B	410	238	Bovine	Foetus	Negative	Negative	Positive	Mild	Severe	Mild
809-12 B	411	76	Ovine	Neonate	Negative	Negative	Negative	Mild	None	None
1669-12 †	412	297	Bovine	Foetus	Negative	-	-	-	-	-

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
525-12	413	365	Ovine	Adult	Negative	Negative	Negative	Severe	None	None
469-12 B	414	98	Ovine	Foetus	Positive	Positive	Positive	Moderate	Severe	None
472-12	415	58	Bovine	Adult	Positive	Positive	Positive	Severe	None	None
1710-12	416	198	Bovine	Neonate	Negative	Negative	Negative	Mild	Mild	None
1651-12	417	353	Ovine	Foetus	Negative	Negative	Positive	Mild	Severe	None
1576-12 B	418	321	Ovine	Foetus	Positive	Positive	Positive	Mild	Mild	None
1654-12	420	167	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Moderate
589-12	421	277	Ovine	Neonate	Positive	Positive	Positive	Mild	Moderate	Moderate
1660-12	422	322	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
556-12	424	246	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
1579-12	425	274	Bovine	Adult	Positive	Negative	Negative	Moderate	None	Mild
585-12 B	426	65	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1670-12 A	427	376	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
1676-12	428	245	Ovine	Neonate	Positive	Positive	Positive	Mild	Severe	None
535-12	429	92	Ovine	Adult	Positive	Positive	Positive	Severe	Moderate	None
599-12	430	162	Bovine	Adult	Positive	Positive	Positive	Mild	Mild	Mild
1737-10	431	388	Caprine	Foetus	Positive	Positive	Positive	Moderate	Moderate	None
611-12	432	449	Ovine	Adult	Positive	Negative	Negative	Moderate	Moderate	None
1049-10 A	433	152	Bovine	Adult	Negative	Negative	Negative	Mild	Moderate	None
822-12	435	185	Ovine	Adult	Positive	Negative	Positive	Severe	Moderate	None
541-12	437	260	Bovine	Adult	Negative	Negative	Negative	Mild	None	Mild
1629-10	438	276	Bovine	Adult	Positive	Negative	Positive	Mild	None	None
474-12	439	418	Caprine	Adult	Negative	Negative	Negative	Mild	None	None
1611-12	440	32	Ovine	Adult	Negative	Negative	Negative	Mild	None	None

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Faculty case number	Random HE number	Random IHC number	Species	Adult or neonate or foetus	rRT-PCR result	IHC result	Histology result	Autolysis	Acid haematin pigment	Bilirubin, haemosideran and/or lipofuchsin
1683-12	441	193	Ovine	Adult	Positive	Negative	Negative	Severe	Mild	None
502-12 A	442	148	Ovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
528-12	443	178	Ovine	Adult	Positive	Negative	Negative	Severe	None	None
1687-12	444	232	Bovine	Neonate	Positive	Positive	Positive	Mild	None	Mild
497-12	445	285	Ovine	Neonate	Positive	Positive	Positive	Mild	None	None
916-10	446	363	Ovine	Foetus	Positive	Positive	Positive	Moderate	None	None
2166-10	447	396	Ovine	Adult	Positive	Positive	Positive	Mild	None	None
557-12	449	279	Ovine	Foetus	Positive	Positive	Positive	Severe	None	Mild
1610-12	450	68	Ovine	Adult	Positive	Positive	Positive	Mild	Mild	Mild
592-12 B	451	379	Ovine	Foetus	Negative	Negative	Negative	Mild	None	Mild
1722-10	452	60	Ovine	Adult	Negative	Negative	Negative	Mild	None	None
550-12	453	146	Bovine	Adult	Positive	Negative	Negative	Mild	None	None
1706-12	455	44	Caprine	Foetus	Negative	Negative	Negative	Mild	None	None
1594-12 B	456	250	Ovine	Foetus	Negative	Negative	Negative	Moderate	None	None
482-12	457	128	Ovine	Foetus	Positive	Negative	Positive	Mild	Severe	None
2147-10	458	143	Ovine	Adult	Positive	Positive	Positive	Mild	Severe	Mild
584-12	460	113	Ovine	Adult	Positive	Positive	Positive	Mild	None	Mild

† Not included in the statistical analysis of the results since liver specimens were absent or could not be conclusively identified in the haematoxylin and eosin stained sections or the immunolabeled sections.

HE = Haematoxylin and eosin. IHC = Immunohistochemistry. rRT-PCR = Real-time reverse-transcriptase polymerase chain reaction.

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APPENDIX 2: Database of all control test cases included in the study.

Controls	Block number	Faculty number	Random HE number	Random IHC number	Actual Disease	IHC antibody applied	Histology result	IHC result
1	09/VH1083	579-09	419	349	RVF	EHV-1	Positive for RVF	Negative for EHV-1
2	09/VH1246	717-09	332	378	RVF	EHV-1	Positive for RVF	Negative for EHV-1
3	09/VH1584	974-09	172	405	RVF	EHV-1	Positive for RVF	Negative for EHV-1
4	09/VH2423	1665-09	61	16	RVF	EHV-1	Positive for RVF	Negative for EHV-1
5	09/VH1345	767-09	454	243	RVF	WBV	Positive for RVF	Negative for RVF
6	09/VH1612	975-09	206	48	RVF	WBV	Positive for RVF	Negative for RVF
7	09/VH1821	1086-09	259	78	RVF	WBV	Positive for RVF	Negative for RVF
8	09/VH1570	937-09	391	130	RVF	WBV	Negative for RVF	Negative for RVF
9	-	883-85	448	393	*Lasiospermum*	RVFV	Negative for RVF	Negative for RVF
10	-	1598-02	335	301	*Xanthuim*	RVFV	Negative for RVF	Negative for RVF
11	-	1599-02	141	70	*Xanthuim*	RVFV	Negative for RVF	Negative for RVF
12	-	1529-09	222	221	*Cestrum*	RVFV	Negative for RVF	Negative for RVF
13	-	3883-09	459	147	Salmonellosis	RVFV	Negative for RVF	Negative for RVF
14	-	604-95	109	429	Seneciosis	RVFV	Negative for RVF	Negative for RVF
15	-	1870-83	253	400	Seneciosis	RVFV	Negative for RVF	Negative for RVF
16	-	1476-10	370	252	*Lantana camara*	RVFV	Negative for RVF	Negative for RVF
17	-	2130-01	12	368	IBR	RVFV	Negative for RVF	Negative for RVF
18	-	2419-01	396	357	IBR	RVFV	Positive for RVF	Negative for RVF
19	-	2753-10 A	312	403	IBR	BHV-1	Positive for RVF	Positive for IBR
20	-	2753-10 B	258	160	IBR	BHV-1	Positive for RVF	Positive for IBR
21	-	1893-09	287	224	EHV-1	EHV-1	Negative for RVF	Positive for EHV-1
22	-	2639-10	254	348	EHV-1	EHV-1	Negative for RVF	Positive for EHV-1
23	-	1869-09	21	30	EHV-1	EHV-1	Negative for RVF	Positive for EHV-1

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Controls	Block number	Faculty number	Random HE number	Random IHC number	Actual Disease	IHC antibody applied	Histology result	IHC result
24	-	2184-11	339	215	EHV-1	EHV-1	Negative for RVF	Positive for EHV-1
25	-	2751-11	82	419	EHV-1	EHV-1	Negative for RVF	Positive for EHV-1
26	07/VH3742	2720-07	243	453	EHV-1	RVFV	Negative for RVF	Negative for RVF
27	07/VH3741	2721-07	380	462	EHV-1	RVFV	Negative for RVF	Negative for RVF
28	09/VH2692	1890-09	25	200	EHV-1	RVFV	Negative for RVF	Negative for RVF
29	09/VH2690	1892-09	257	175	EHV-1	RVFV	Negative for RVF	Negative for RVF
30	09/VH2486	1775-09	197	88	EHV-1	RVFV	Negative for RVF	Negative for RVF
31	-	1713-10	358	406	WBD	WBV	Negative for RVF	Positive for WBD
32	-	2979-10	50	432	WBD	WBV	Negative for RVF	Positive for WBD
33	-	1248-11	247	359	WBD	WBV	Negative for RVF	Positive for WBD
34	PC/11-4797 B	1484-11 B	145	442	WBD	WBV	Negative for RVF	Positive for WBD
35	-	2166-11 A	140	404	WBD	WBV	Negative for RVF	Positive for WBD
36	PC/11-3477	1149-11	423	392	WBD	RVFV	Positive for RVF	Negative for RVF
37	PC/11-4797 A	1484-11 A	436	86	WBD	RVFV	Negative for RVF	Negative for RVF
38	PC/11-066	597-11	157	228	WBD	RVFV	Negative for RVF	Negative for RVF
39	-	2166-11 B	41	46	WBD	RVFV	Negative for RVF	Negative for RVF
40	-	2166-11 C	434	133	WBD	RVFV	Negative for RVF	Negative for RVF

HE = Haematoxylin and eosin. IHC = Immunohistochemistry.

RVF = Rift Valley fever. RVFV = Rift Valley fever virus.

IBR = Infectious bovine rhinotracheitis. BHV-1 = Bovine herpesvirus 1.

EHV-1 = Equine herpesvirus 1.

WBD = Wesselsbron disease. WBV = Wesselsbron virus.

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APPENDIX 3: Table indicating histomorphological features that were examined for each haematoxylin and eosin stained slide. (The original Excel document is depicted in a JPG format below.)

Liver criterion								Comments
Vacuolation hepatocytes	*	Steatosis	microvesicular	macrovesicular	mixed	Hydropic		
	*	mild	moderate	severe				
	*	Periacinar	midzonal	periportal	mixed			
Portal tracts	*	Bile duct necrosis	BD hyperplasia	limiting plate prolif activity				
	*	Lesion in portal vein	hepatic artery	lymphatics	interstitium			
	*	Inflammation	ducts	interstitium	neutrophils	lymphoid cells		
		macrophages	eosinophils	necrotic infl cells				
	*	Oedema	fibrosis	dilated lymphatics				
	*	Lesion in limiting plate & portal hepatocytes	portal triads preserved					
	*	Arteriolar proliferation						
Adaptive responses	*	atrophy	regenerative nodules	cirrhosis				
Fibrosis	*	periacinar	biliary	diffuse	post-necrotic			
Cholestasis	*	hepatocellular	canaliculi	Kupffer cells	bile ductules			
Kupffer cells	*	swollen	proliferation	apoptotic	haemosiderin	phagocytosed bile plugs	eosinophilic cytoplasmic bodies	
		localized response	widespread response	pyknosis				
Ito cells	*	Inapparent	prominent	hyperplasia	hypertrophy			
Pigments	*	bile	haemosiderin	lipofuscin	melanin	acid formaldehyde hematin		
	*	mild	moderate	severe				
Vascular lesions (PA and other, not portal)	*	lymphatics (also capsule)	veins	arteries	sinusoids			
		vasculitis	segmental	complete				
	*	endothelial cell pathology	necrosis	hyperplasia	swelling	sparse		
	*	fibrin thrombi	assoc with necrotic bv	shock bodies/fibrin droplets/sinusoidal acidophilic bodies				
		periacinar	midzonal	periportal	CV	Portal vessels		
Other lesions		abscesses	Granulomas	Other				

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Liver criterion								Comments
Autolysis		mild	moderate	severe	none			
EMH		mild	moderate	severe	none			
Liver necrosis	*	mild	moderate	severe				
	*	Coagulative	lytic	ischaemic				
	*	Apoptosis	mild	moderate	severe	PA		
		MZ	PP	random				
	*	single cell	focal or multifocal	small (up to 10 hepatocytes)		large		
		coalescent	discrete	random	zonal (PA/ MZ/ periportal)			
		1/3 lobule	confluent bridging	2/3 lobule or extensive	pan-necrosis	only reticulum network		
	*	primary coag foci superimposed on pan-necrosis			random	well-circumscribed		
		multifocal	sparse	widespread	Coalescent	Discreet		
	*	central veins occluded by necrotic foci			Variable			
	*	karyorrhexis	karyolysis	pycnosis				
	*	Hypereosinophilic cyto		eosinophilic cytoplasmic bodies		hepatocyte individualisation & disassociation		
	*	Mineralization of single cells	groups of necrotic hepatocytes	mild	moderate	severe	in primary necrotic foci	
	*	Haemorrhage in necrotic foci	blood pooling in necrotic areas		sinusoidal distension	disseminated haemorrhages	mild	
		moderate	severe	PA	MZ	PP		
	*	Inflamm in necrotic foci	neutrophils	lymphoid cells	macro-phages	sinusoidal		
		pycnosis	karyorrhexis	karyolysis	PA	MZ	PP	
Con-gestion	*	Periacinar	midzonal	periportal				
	*	Mild	Moderate	Severe				
Nuclear changes Inclusion bodies	*	Anisonucleo-sis & ka-ryomegaly	mild	moderate	severe	empty, ballooned nuclear shells	vesicular nuclei	
		chromatin fragmentation	nuclear membrane-assoc chromatin		blocky chromatin	coarse chromatin		
	*	intranuclear	intracytoplas					
	*	eosinophilic	basophilic	amphophilic				
	*	Large	medium	small IB				
	*	Filamentous	cigar-shaped	rod-shaped	oval	elongated		
		round	ring-shaped	pleomorphic				
	*	Single	multiple					
	*	central	peripheral location					
	*	Distinct	indistinct					
	*	Associated with chromatin margination						
	*	@edges of necrotic foci	in necrotic foci	widespread				

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APPENDIX 4: Form on which the randomly assigned number, a summarized description of the lesions, and the results for each haematoxylin and eosin stained slide were recorded and later correlated with the original case number once all the cases had been viewed. (The original Excel document is depicted in a JPG format below.)

Randomly assigned number:	
Idexx case number:	
OP case number:	

Comments on HE slide:

EMH:	None	Mild	Moderate	Severe
Autolysis:	None	Mild	Moderate	Severe
Acid haematin pigment:	None	Mild	Moderate	Severe
Other pigments:	None	Bilirubin	Haemosiderin	Lipofuscin

Liver lesions:	Consistent with RVF	Not consistent with RVF

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APPENDIX 5: Table indicating histomorphological features that were examined for each immunohistochemically labelled slide. (The original Excel document is depicted in a JPG format below.)

Liver criterion							Comments
Necrotic foci or areas	*	single cell	focal or multifocal	small (up to 10 hepatocytes)	large		
		coalescent	discrete	random	zonal (PA/ MZ/ periportal)		
		1/3 lobule	confluent bridging	2/3 lobule or extensive	massive		
	*	cytoplasmic	nuclear	inclusions	pseudo-inclusions		
	*	amount of necrosis exceeds the amount of positive staining			mild	moderate	severe
	*	individualised rounded necrotic cells		dense granular	sparse granular	diffuse pale	
		necrotic cytoplasmic remnants		fine granules	course granules		
	*	Kuffer cells	mononuclear	dense granular	sparse granular	diffuse pale	
		neutrophils	lymphoid	fine granules	course granules		
	*	delineating cytoplasmic margins		morula-like structures			
	*	delineating the sinusoidal spaces / reticulum framework		beads	linear punctate dots		
Prim foci	*	necrotic cytoplasmic remnants		dense granular	sparse granular	diffuse pale	
		Kupffer cells	mononuclear	fine granules	course granules	morula-like structures	
		neutrophils	lymphoid				
Sinusoids	*	delineating the sinusoidal spaces / reticulum framework		beads	linear punctate dots		
	*	Kupffer cells	mononuclear	dense granular	sparse granular	diffuse pale	
		lymphoid		fine granules	course granules		
	*	endothelial		beads	linear punctate dots		
	*	morula-like structures					
Portal tracts	*	limiting plate	portal hepatocytes	limiting plate prolif cells	BD hyperplastic		
		hepatic artery	portal vein	lymphatics	bile duct		
	*	endothelial	smooth muscle	epithelial			
	*	macrophage	monocyte	neutrophils	lymphoid		
	*	necrotic cytoplasmic remnants		dense granular	sparse granular	diffuse pale	
		individualised rounded necrotic cells		fine granules	course granules		

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APPENDIX 6: Form on which the randomly assigned numbers, additional comments, and the results of each immunohistochemically labelled slide were recorded and later correlated with the original case number once all the cases had been viewed. (The original Excel document is depicted in a JPG format below.)

Randomly assigned number:	
Idexx case number:	
OP case number:	
Date collected:	

Comments on IMP slide:

IMP	Positive	Negative
PCR	Positive	Negative

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APPENDIX 7: Program code for the conditional independence model used in WinBUGS with informative priors for histopathology and rRT-PCR and uninformative priors for prevalence and IHC.

```
model;
{
# define multinomial distribution

# y is the number of populations
# p is the probability estimate for a multinomial cell in a 2x4 array for three tests
# n is the total number of individuals sampled in each population
# Pr is the prevalence of the disease in each population
# Se is the sensitivity of each of the three tests
# Sp is the specificity of each of the three tests

# Test order PCR = 1, IHC = 2, Histo = 3

y1[1:8] ~ dmulti(p1[1:8], n1)
p1[1] < - Pr1*Se1*Se2*Se3 + (1-Pr1)*(1-Sp1)*(1-Sp2)*(1-Sp3)
p1[2] < - Pr1*Se1*(1-Se2)*Se3 + (1-Pr1)*(1-Sp1)*Sp2*(1-Sp3)
p1[3] < - Pr1*Se1*Se2*(1-Se3) + (1-Pr1)*(1-Sp1)*(1-Sp2)*Sp3
p1[4] < - Pr1*Se1*(1-Se2)*(1-Se3) + (1-Pr1)*(1-Sp1)*Sp2*Sp3
p1[5] < - Pr1*(1-Se1)*Se2*Se3 + (1-Pr1)*Sp1*(1-Sp2)*(1-Sp3)
p1[6] < - Pr1*(1-Se1)*(1-Se2)*Se3 + (1-Pr1)*Sp1*Sp2*(1-Sp3)
p1[7] < - Pr1*(1-Se1)* Se2*(1-Se3) + (1-Pr1)*Sp1*(1-Sp2)*Sp3
p1[8] < - Pr1*(1-Se1)*(1-Se2)*(1-Se3) + (1-Pr1)*Sp1*Sp2*Sp3

# prior probability distributions

Pr1 ~ dbeta (1,1)
Se1 ~ dbeta (107,2.5)      # Se for PCR   = 98.0% and 90% PI [99.5, 95.0]
Sp1 ~ dbeta (12,3.3)       # Sp for PCR   = 79.7% and 90% PI [59.7, 92.8]
Se2 ~ dbeta (1,1)          # Se for IHC    = 50.0% and 90% PI [5.0, 95.0]
Sp2 ~ dbeta (1,1)          # Sp for IHC    = 50.0% and 90% PI [5.0, 95.0]
Se3 ~ dbeta (6.9,1.1)      # Se for Histo  = 89.2% and 90% PI [63.2, 99.0]
Sp3 ~ dbeta (18.45,3.55)  # Sp for Histo  = 84.9% and 90% PI [69.7, 94.5]
}

#data
list(n1=380,y1=c(188,8,11,50,6,9,0,124))
```


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APPENDIX 8: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the unstratified data set, and the data stratified into species (cattle or sheep), age category (adult, neonate or foetus), level of autolysis (mild, moderate or severe) and the presence or absence of tissue pigments (haemosiderin, bilirubin or lipofuchsin).

In all the tables:

rRT-PCR = Real-time reverse-transcriptase polymerase chain reaction.

Histo = Histopathology. IHC = Immunohistochemistry

APPENDIX 8.1: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the unstratified data set

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	41	0	2	1	44
	T^{-} (Histo)	3	7	0	18	28
	Totals:	44	7	2	19	72

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APPENDIX 8.2: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for all bovine animals.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	57	1	2	4	64
	T^{-} (Histo)	2	19	0	34	55
	Totals:	59	20	2	38	119

APPENDIX 8.3: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for all ovine animals.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	131	7	4	5	147
	T^{-} (Histo)	9	31	0	74	114
	Totals:	140	38	4	79	261

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APPENDIX 8.4: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for adult ovine and bovine animals.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	87	6	3	3	101
	T^{-} (Histo)	6	30	0	66	102
	Totals:	93	36	3	69	201

APPENDIX 8.5: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for ovine and bovine neonates.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	45	0	2	1	48
	T^{-} (Histo)	2	14	0	21	37
	Totals:	47	14	2	22	85

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APPENDIX 8.6: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for ovine and bovine foetuses.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	56	2	1	5	64
	T^{-} (Histo)	3	6	0	21	30
	Totals:	59	8	1	26	94

APPENDIX 8.7: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for mildly autolysed specimens.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	T^{+}(Histo)	127	2	3	6	138
	T^{-} (Histo)	6	24	0	75	105
	Totals:	133	26	3	81	243

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APPENDIX 8.8: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for moderately autolysed specimens.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	$T^{+(Histo)}$	41	0	2	1	44
	T^{-} (Histo)	3	7	0	18	28
	Totals:	44	7	2	19	72

APPENDIX 8.9: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for severely autolysed specimens.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	$T^{+(Histo)}$	20	6	1	2	29
	T^{-} (Histo)	2	19	0	15	36
	Totals:	22	25	1	17	65

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APPENDIX 8.10: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for cases where tissue pigments were present.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	$T^{+(Histo)}$	50	3	1	4	58
	T^{-} (Histo)	3	13	0	26	42
	Totals:	53	16	1	30	100

APPENDIX 8.11: Cross-classified test outcomes from rRT-PCR, histopathology, and IHC with respect to the data for cases where tissue pigments were absent.

	Histo	rRT-PCR				
		T^{+} (rRT-PCR)		T^{-} (rRT-PCR)		
		T^{+} (IHC)	T^{-} (IHC)	T^{+} (IHC)	T^{-} (IHC)	
	$T^{+(Histo)}$	138	5	5	5	153
	T^{-} (Histo)	8	37	0	82	127
	Totals:	146	42	5	87	280

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Appendix 9: Analysis of diagnostic test accuracy of the data set using informative priors for histopathology and rRT-PCR, and uninformative priors for prevalence and IHC. Depicted are: Posterior values obtained for each parameter sampled every 20 000 iterations and at different starting points; Plots of iterates for each variable to check for convergence of the monitored model parameters; Kernel density estimate and; Autocorrelation plots.

Appendix 9.1: Analysis of the unstratified data set for the 380 ovine and bovine animals.

Appendix 9.1.1: Posterior values obtained for each parameter for the unstratified data set for the ovine and bovine animals.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5274	0.02581	1.31E-04	0.477	0.5272	0.5782	101	39800	0.53 [0.48, 0.58]
Se1	0.9732	0.00919	4.96E-05	0.9526	0.9742	0.9882	101	39800	0.97 [0.95, 0.99]
Se2	0.974	0.01545	8.06E-05	0.9388	0.9759	0.9977	101	39800	0.98 [0.94, 0.99]
Se3	0.9448	0.01612	8.03E-05	0.9096	0.9461	0.9724	101	39800	0.95 [0.91, 0.97]
Sp1	0.7166	0.03272	1.63E-04	0.6504	0.7172	0.7786	101	39800	0.72 [0.65, 0.78]
Sp2	0.9916	0.00833	4.11E-05	0.9691	0.9942	0.9998	101	39800	0.99 [0.97, 0.99]
Sp3	0.9211	0.02093	1.06E-04	0.8762	0.9225	0.9576	101	39800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5274	0.02577	8.99E-05	0.4769	0.5274	0.5779	101	79800	0.53 [0.48, 0.58]
Se1	0.9732	0.00915	3.35E-05	0.9527	0.9742	0.9882	101	79800	0.97 [0.95, 0.99]
Se2	0.9739	0.01539	5.35E-05	0.9392	0.9757	0.9977	101	79800	0.98 [0.94, 0.99]
Se3	0.9448	0.01617	5.57E-05	0.9094	0.9461	0.9723	101	79800	0.95 [0.91, 0.97]
Sp1	0.7166	0.03276	1.15E-04	0.6503	0.7173	0.7789	101	79800	0.72 [0.65, 0.78]
Sp2	0.9916	0.00836	3.01E-05	0.969	0.9942	0.9998	101	79800	0.99 [0.97, 0.99]
Sp3	0.9212	0.02092	7.30E-05	0.8763	0.9226	0.9577	101	79800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5274	0.02582	7.24E-05	0.4768	0.5275	0.5781	101	119800	0.53 [0.48, 0.58]
Se1	0.9732	0.00915	2.76E-05	0.9527	0.9742	0.9882	101	119800	0.97 [0.95, 0.99]
Se2	0.9739	0.0154	4.50E-05	0.9392	0.9757	0.9977	101	119800	0.98 [0.94, 0.99]
Se3	0.9448	0.01613	4.56E-05	0.9095	0.9462	0.9723	101	119800	0.95 [0.91, 0.97]
Sp1	0.7165	0.03279	9.25E-05	0.6502	0.7172	0.7789	101	119800	0.72 [0.65, 0.78]
Sp2	0.9916	0.00835	2.43E-05	0.969	0.9942	0.9998	101	119800	0.99 [0.97, 0.99]
Sp3	0.9212	0.0209	5.77E-05	0.8764	0.9227	0.9576	101	119800	0.92 [0.88, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5274	0.02579	6.50E-05	0.4769	0.5275	0.5781	101	159800	0.53 [0.48, 0.58]
Se1	0.9733	0.00913	2.31E-05	0.9528	0.9742	0.9882	101	159800	0.97 [0.95, 0.99]
Se2	0.9739	0.01541	3.85E-05	0.9392	0.9757	0.9977	101	159800	0.98 [0.94, 0.99]
Se3	0.9448	0.01613	3.99E-05	0.9094	0.9462	0.9723	101	159800	0.95 [0.91, 0.97]
Sp1	0.7165	0.03282	8.30E-05	0.6501	0.7172	0.7789	101	159800	0.72 [0.65, 0.78]
Sp2	0.9916	0.00833	2.11E-05	0.9691	0.9942	0.9998	101	159800	0.99 [0.97, 0.99]
Sp3	0.9212	0.02091	5.10E-05	0.8764	0.9227	0.9576	101	159800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.0258	5.71E-05	0.4768	0.5276	0.5781	101	199800	0.53 [0.48, 0.58]
Se1	0.9733	0.00913	2.05E-05	0.9527	0.9743	0.9882	101	199800	0.97 [0.95, 0.99]
Se2	0.9739	0.01542	3.43E-05	0.9391	0.9757	0.9977	101	199800	0.98 [0.94, 0.99]
Se3	0.9448	0.01611	3.50E-05	0.9095	0.9462	0.9723	101	199800	0.95 [0.91, 0.97]
Sp1	0.7164	0.03278	7.23E-05	0.6502	0.7172	0.7787	101	199800	0.72 [0.65, 0.78]
Sp2	0.9916	0.00832	1.85E-05	0.9692	0.9942	0.9998	101	199800	0.99 [0.97, 0.99]
Sp3	0.9212	0.02094	4.66E-05	0.8763	0.9227	0.9576	101	199800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02581	5.78E-05	0.4768	0.5276	0.5781	20000	200002	0.53 [0.48, 0.58]
Se1	0.9733	0.00915	1.97E-05	0.9526	0.9743	0.9882	20000	200002	0.97 [0.95, 0.99]
Se2	0.9739	0.01541	3.33E-05	0.9392	0.9757	0.9977	20000	200002	0.98 [0.94, 0.99]
Se3	0.9448	0.0161	3.52E-05	0.9095	0.9462	0.9723	20000	200002	0.95 [0.91, 0.97]
Sp1	0.7164	0.03274	7.22E-05	0.6502	0.7172	0.7787	20000	200002	0.72 [0.65, 0.78]
Sp2	0.9916	0.00834	1.90E-05	0.9692	0.9941	0.9998	20000	200002	0.99 [0.97, 0.99]
Sp3	0.9212	0.02093	4.55E-05	0.8762	0.9227	0.9577	20000	200002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02583	6.77E-05	0.4769	0.5276	0.5782	40000	160002	0.53 [0.48, 0.58]
Se1	0.9733	0.00916	2.23E-05	0.9526	0.9743	0.9882	40000	160002	0.97 [0.95, 0.99]
Se2	0.9739	0.01543	3.81E-05	0.939	0.9757	0.9977	40000	160002	0.98 [0.94, 0.99]
Se3	0.9448	0.01607	3.84E-05	0.9096	0.9462	0.9723	40000	160002	0.95 [0.91, 0.97]
Sp1	0.7164	0.03273	8.43E-05	0.6502	0.7171	0.7786	40000	160002	0.72 [0.65, 0.78]
Sp2	0.9916	0.00833	2.18E-05	0.9692	0.9941	0.9998	40000	160002	0.99 [0.97, 0.99]
Sp3	0.9212	0.02094	5.05E-05	0.8762	0.9227	0.9576	40000	160002	0.92 [0.88, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02579	8.01E-05	0.4769	0.5276	0.5781	60000	120002	0.53 [0.48, 0.58]
Se1	0.9733	0.00917	2.59E-05	0.9526	0.9743	0.9882	60000	120002	0.97 [0.95, 0.99]
Se2	0.9739	0.01544	4.28E-05	0.939	0.9757	0.9978	60000	120002	0.98 [0.94, 0.99]
Se3	0.9448	0.01608	4.54E-05	0.9096	0.9462	0.9723	60000	120002	0.95 [0.91, 0.97]
Sp1	0.7164	0.03269	9.72E-05	0.6503	0.7172	0.7785	60000	120002	0.72 [0.65, 0.78]
Sp2	0.9916	0.00833	2.48E-05	0.9693	0.9942	0.9998	60000	120002	0.99 [0.97, 0.99]
Sp3	0.9211	0.02096	5.92E-05	0.876	0.9227	0.9577	60000	120002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02584	9.92E-05	0.4769	0.5275	0.5781	80000	80002	0.53 [0.48, 0.58]
Se1	0.9732	0.00922	3.10E-05	0.9524	0.9742	0.9882	80000	80002	0.97 [0.95, 0.99]
Se2	0.9739	0.01544	5.52E-05	0.9389	0.9757	0.9978	80000	80002	0.98 [0.94, 0.99]
Se3	0.9448	0.01606	5.32E-05	0.9098	0.9462	0.9723	80000	80002	0.95 [0.91, 0.97]
Sp1	0.7165	0.03258	1.13E-04	0.6506	0.7172	0.7783	80000	80002	0.72 [0.65, 0.78]
Sp2	0.9916	0.00836	2.98E-05	0.9692	0.9942	0.9998	80000	80002	0.99 [0.97, 0.99]
Sp3	0.9211	0.02099	7.22E-05	0.8758	0.9226	0.9578	80000	80002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02586	1.42E-04	0.4772	0.5273	0.5784	100000	40002	0.53 [0.48, 0.58]
Se1	0.9732	0.00929	4.25E-05	0.9521	0.9742	0.9882	100000	40002	0.97 [0.95, 0.99]
Se2	0.9739	0.0154	7.52E-05	0.9392	0.9756	0.9978	100000	40002	0.98 [0.94, 0.99]
Se3	0.9448	0.01609	7.86E-05	0.9096	0.946	0.9724	100000	40002	0.95 [0.91, 0.97]
Sp1	0.7166	0.03256	1.60E-04	0.6508	0.7172	0.7788	100000	40002	0.72 [0.65, 0.78]
Sp2	0.9915	0.00845	4.25E-05	0.9688	0.9941	0.9998	100000	40002	0.99 [0.97, 0.99]
Sp3	0.9212	0.02092	1.06E-04	0.8759	0.9227	0.9578	100000	40002	0.92 [0.88, 0.96]

Pr1 = Prevalence of disease in the population.

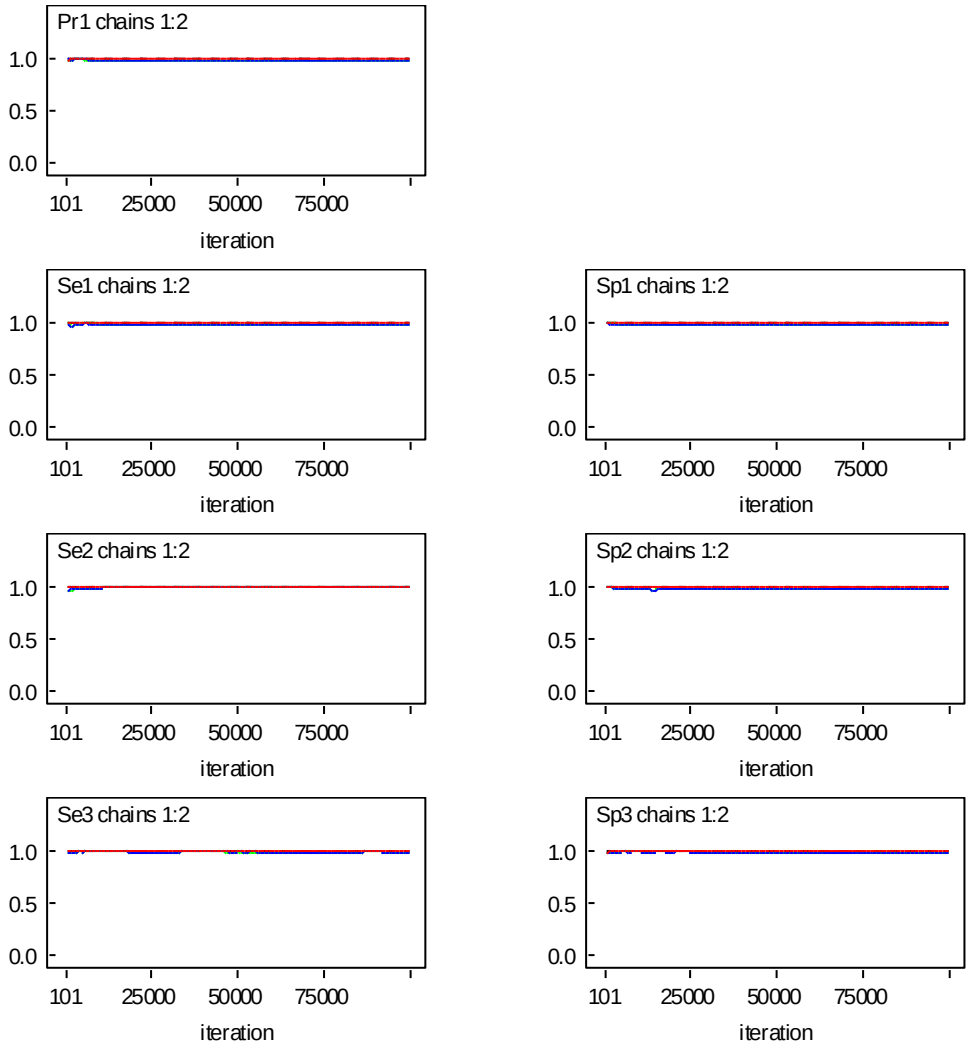
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.1.2: Plots of iterates for each variable to check for convergence for the unstratified data set for the ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

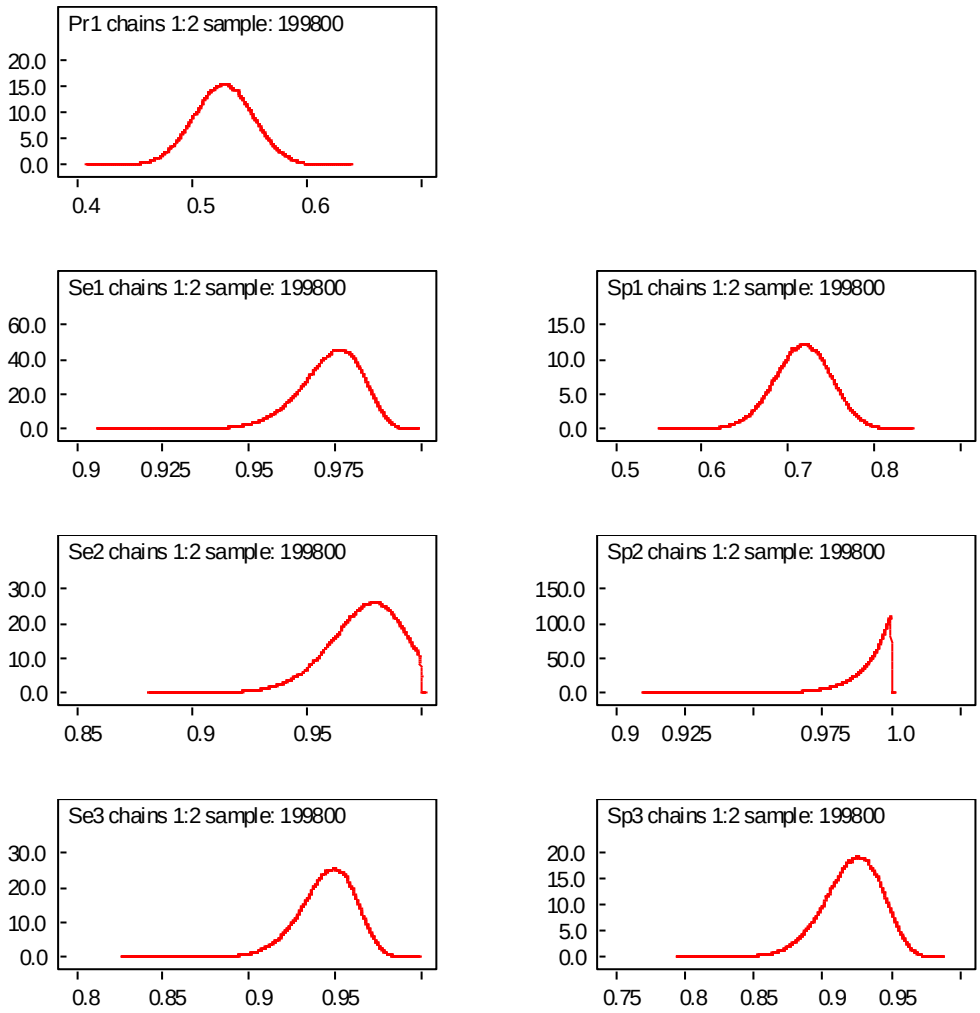
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.1.3: Kernel density estimate for the unstratified data set for the ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

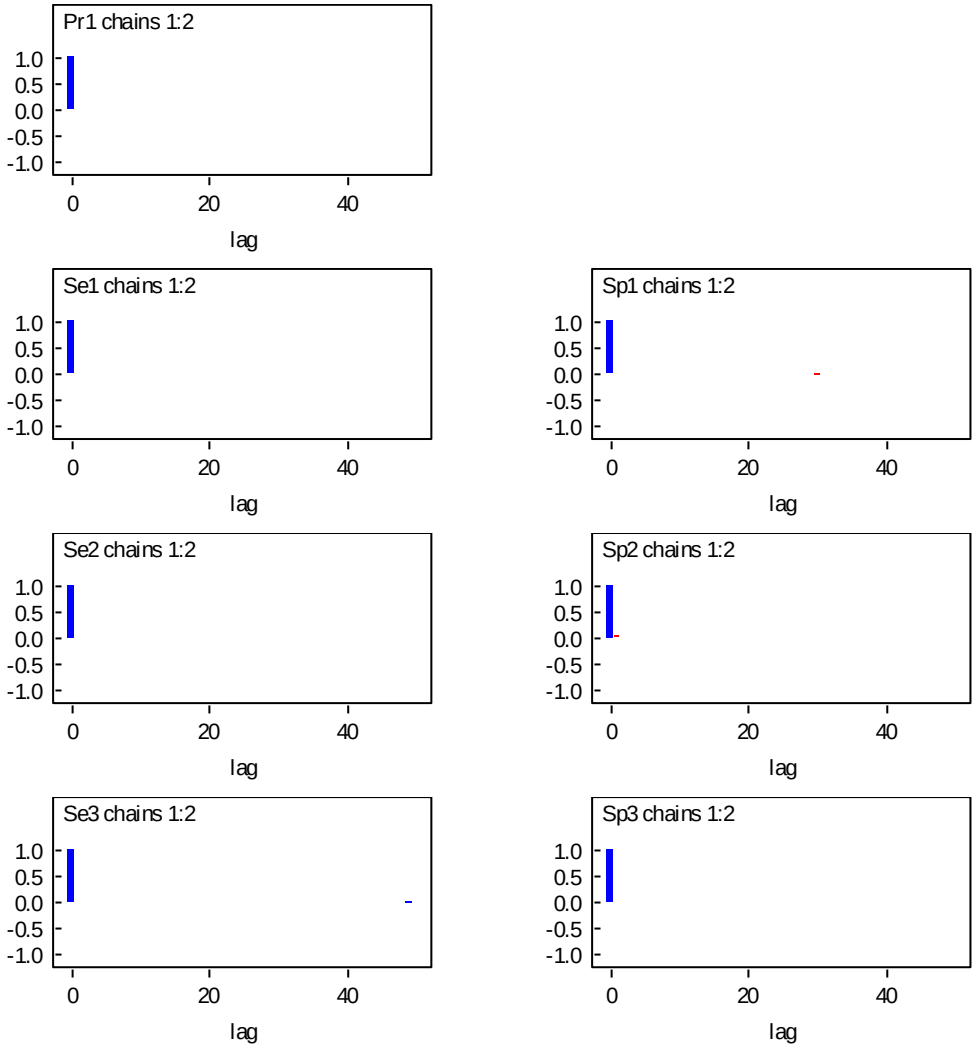
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.1.4: Autocorrelation plots for the unstratified data set for the ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.2: Analysis of the data set for the 119 bovine animals.

Appendix 9.2.1: Posterior values obtained for each parameter for the data set for bovine animals.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5115	0.04627	2.51E-04	0.4204	0.5115	0.6028	101	39800	0.57 [0.50, 0.63]
Se1	0.9743	0.01244	5.82E-05	0.9453	0.9762	0.993	101	39800	0.98 [0.95, 0.99]
Se2	0.9766	0.02114	1.07E-04	0.9215	0.9824	0.9993	101	39800	0.96 [0.91, 0.99]
Se3	0.9597	0.02541	1.31E-04	0.899	0.9641	0.9957	101	39800	0.94 [0.89, 0.97]
Sp1	0.683	0.05439	2.75E-04	0.5716	0.6846	0.7845	101	39800	0.71 [0.63, 0.79]
Sp2	0.9732	0.02467	1.32E-04	0.9083	0.9804	0.9992	101	39800	0.99 [0.95, 0.99]
Sp3	0.8957	0.03493	1.84E-04	0.8177	0.8995	0.9534	101	39800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5113	0.04632	1.62E-04	0.4203	0.5113	0.6021	101	79800	0.57 [0.50, 0.63]
Se1	0.9743	0.01246	4.41E-05	0.9451	0.9762	0.993	101	79800	0.98 [0.95, 0.99]
Se2	0.9765	0.02111	7.98E-05	0.9213	0.9824	0.9993	101	79800	0.96 [0.92, 0.99]
Se3	0.9598	0.02543	8.83E-05	0.8988	0.9643	0.9957	101	79800	0.94 [0.89, 0.97]
Sp1	0.6829	0.05443	1.94E-04	0.5708	0.6845	0.784	101	79800	0.71 [0.63, 0.79]
Sp2	0.9732	0.02468	9.19E-05	0.9084	0.9803	0.9992	101	79800	0.99 [0.95, 0.99]
Sp3	0.8958	0.03483	1.27E-04	0.8183	0.8994	0.9538	101	79800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5113	0.04631	1.33E-04	0.4204	0.5113	0.602	101	119800	0.57 [0.50, 0.63]
Se1	0.9743	0.01243	3.61E-05	0.9451	0.9762	0.993	101	119800	0.98 [0.95, 0.99]
Se2	0.9765	0.0212	6.68E-05	0.9211	0.9824	0.9993	101	119800	0.96 [0.91, 0.99]
Se3	0.9598	0.02542	7.17E-05	0.8986	0.9643	0.9956	101	119800	0.94 [0.89, 0.97]
Sp1	0.6829	0.05455	1.61E-04	0.5708	0.6846	0.7845	101	119800	0.71 [0.63, 0.79]
Sp2	0.9732	0.02472	7.50E-05	0.9081	0.9804	0.9993	101	119800	0.99 [0.95, 0.99]
Sp3	0.8957	0.03488	1.07E-04	0.8182	0.8992	0.9538	101	119800	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5114	0.04623	1.15E-04	0.4208	0.5114	0.6019	101	159800	0.57 [0.50, 0.63]
Se1	0.9743	0.01242	3.01E-05	0.9451	0.9762	0.993	101	159800	0.98 [0.95, 0.99]
Se2	0.9765	0.02119	5.55E-05	0.921	0.9824	0.9993	101	159800	0.96 [0.91, 0.99]
Se3	0.9598	0.0254	6.12E-05	0.8988	0.9643	0.9956	101	159800	0.94 [0.89, 0.97]
Sp1	0.6827	0.05445	1.39E-04	0.571	0.6844	0.7844	101	159800	0.71 [0.63, 0.79]
Sp2	0.9732	0.02473	6.57E-05	0.908	0.9804	0.9993	101	159800	0.99 [0.95, 0.99]
Sp3	0.8958	0.03496	8.76E-05	0.8181	0.8993	0.9539	101	159800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	
Pr1	0.5113	0.04623	1.04E-04	0.4207	0.5113	0.6016	101	199800	0.57 [0.50, 0.63]
Se1	0.9743	0.0124	2.64E-05	0.9453	0.9762	0.993	101	199800	0.98 [0.95, 0.99]
Se2	0.9765	0.02119	4.79E-05	0.921	0.9824	0.9993	101	199800	0.96 [0.91, 0.99]
Se3	0.9599	0.02538	5.46E-05	0.8989	0.9643	0.9956	101	199800	0.94 [0.89, 0.97]
Sp1	0.6827	0.05445	1.28E-04	0.5711	0.6844	0.7844	101	199800	0.71 [0.63, 0.79]
Sp2	0.9731	0.02479	5.76E-05	0.9081	0.9803	0.9993	101	199800	0.99 [0.95, 0.99]
Sp3	0.8958	0.03496	8.04E-05	0.818	0.8993	0.9538	101	199800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5112	0.04621	1.06E-04	0.4207	0.5112	0.6013	20000	200002	0.57 [0.50, 0.63]
Se1	0.9744	0.01238	2.61E-05	0.9453	0.9762	0.993	20000	200002	0.98 [0.95, 0.99]
Se2	0.9765	0.02121	4.84E-05	0.9209	0.9824	0.9993	20000	200002	0.96 [0.91, 0.99]
Se3	0.9599	0.02538	5.53E-05	0.8988	0.9645	0.9955	20000	200002	0.94 [0.89, 0.97]
Sp1	0.6826	0.05453	1.28E-04	0.5708	0.6843	0.7845	20000	200002	0.71 [0.63, 0.79]
Sp2	0.9731	0.02481	5.66E-05	0.908	0.9803	0.9993	20000	200002	0.99 [0.95, 0.99]
Sp3	0.8957	0.03491	8.22E-05	0.8182	0.8992	0.9539	20000	200002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5112	0.04616	1.18E-04	0.4208	0.5112	0.6012	40000	160002	0.57 [0.50, 0.63]
Se1	0.9744	0.01236	2.89E-05	0.9453	0.9762	0.993	40000	160002	0.98 [0.95, 0.99]
Se2	0.9765	0.02124	5.36E-05	0.9209	0.9825	0.9993	40000	160002	0.96 [0.91, 0.99]
Se3	0.9599	0.02536	6.27E-05	0.8989	0.9645	0.9955	40000	160002	0.94 [0.89, 0.97]
Sp1	0.6826	0.05454	1.37E-04	0.571	0.6843	0.7847	40000	160002	0.71 [0.63, 0.79]
Sp2	0.9731	0.02484	6.27E-05	0.9079	0.9803	0.9993	40000	160002	0.99 [0.95, 0.99]
Sp3	0.8957	0.03496	9.16E-05	0.8181	0.8991	0.9538	40000	160002	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5111	0.04612	1.35E-04	0.4209	0.5112	0.6009	60000	120002	0.57 [0.50, 0.63]
Se1	0.9744	0.01236	3.49E-05	0.9455	0.9762	0.993	60000	120002	0.98 [0.95, 0.99]
Se2	0.9766	0.02121	5.99E-05	0.921	0.9825	0.9993	60000	120002	0.96 [0.91, 0.99]
Se3	0.96	0.02535	7.18E-05	0.899	0.9645	0.9956	60000	120002	0.94 [0.89, 0.97]
Sp1	0.6825	0.05446	1.56E-04	0.571	0.6842	0.7845	60000	120002	0.71 [0.63, 0.79]
Sp2	0.9731	0.02486	7.02E-05	0.908	0.9803	0.9993	60000	120002	0.99 [0.95, 0.99]
Sp3	0.8957	0.03495	1.06E-04	0.8182	0.8992	0.9538	60000	120002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5109	0.0462	1.69E-04	0.4202	0.511	0.6007	80000	80002	0.57 [0.50, 0.63]
Se1	0.9744	0.01234	3.98E-05	0.9455	0.9762	0.993	80000	80002	0.98 [0.95, 0.99]
Se2	0.9766	0.02122	7.58E-05	0.9211	0.9825	0.9993	80000	80002	0.96 [0.91, 0.99]
Se3	0.96	0.02536	8.91E-05	0.8989	0.9646	0.9956	80000	80002	0.94 [0.89, 0.97]
Sp1	0.6826	0.05461	1.87E-04	0.5709	0.6843	0.7846	80000	80002	0.71 [0.63, 0.79]
Sp2	0.973	0.02491	8.55E-05	0.908	0.9803	0.9992	80000	80002	0.99 [0.95, 0.99]
Sp3	0.8957	0.03482	1.33E-04	0.8184	0.8991	0.9535	80000	80002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5109	0.04616	2.43E-04	0.4205	0.511	0.601	100000	40002	0.57 [0.50, 0.63]
Se1	0.9744	0.01237	5.69E-05	0.9453	0.9762	0.9931	100000	40002	0.98 [0.95, 0.99]
Se2	0.9766	0.02127	1.05E-04	0.9211	0.9825	0.9993	100000	40002	0.96 [0.91, 0.99]
Se3	0.9599	0.02543	1.26E-04	0.8985	0.9646	0.9955	100000	40002	0.94 [0.89, 0.97]
Sp1	0.6827	0.0548	2.64E-04	0.5701	0.6845	0.7849	100000	40002	0.71 [0.63, 0.79]
Sp2	0.9731	0.02478	1.25E-04	0.9079	0.9803	0.9992	100000	40002	0.99 [0.95, 0.99]
Sp3	0.8956	0.03469	1.90E-04	0.819	0.8988	0.9536	100000	40002	0.92 [0.86, 0.96]

Pr1 = Prevalence of disease in the population.

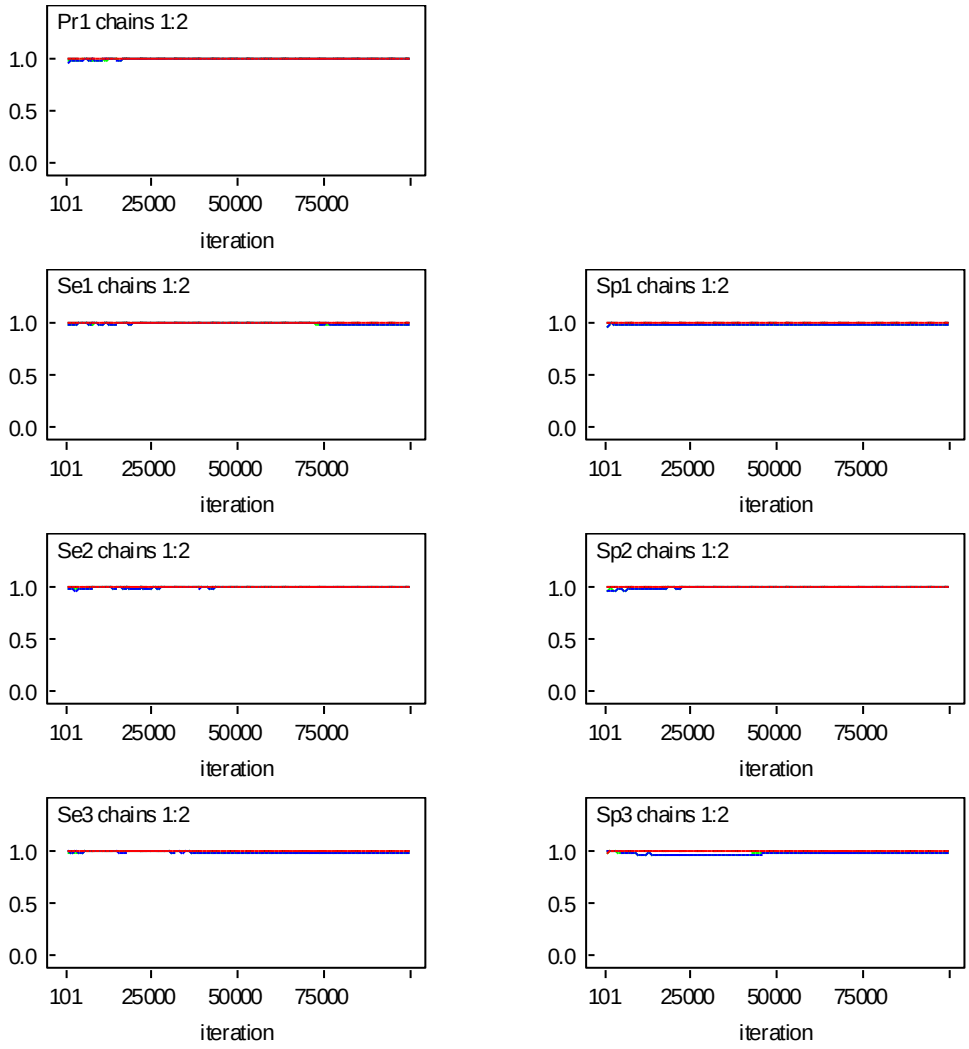
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.2.2: Plots of iterates for each variable to check for convergence for the data set for bovine animals.



Pr1 = Prevalence of disease in the population.

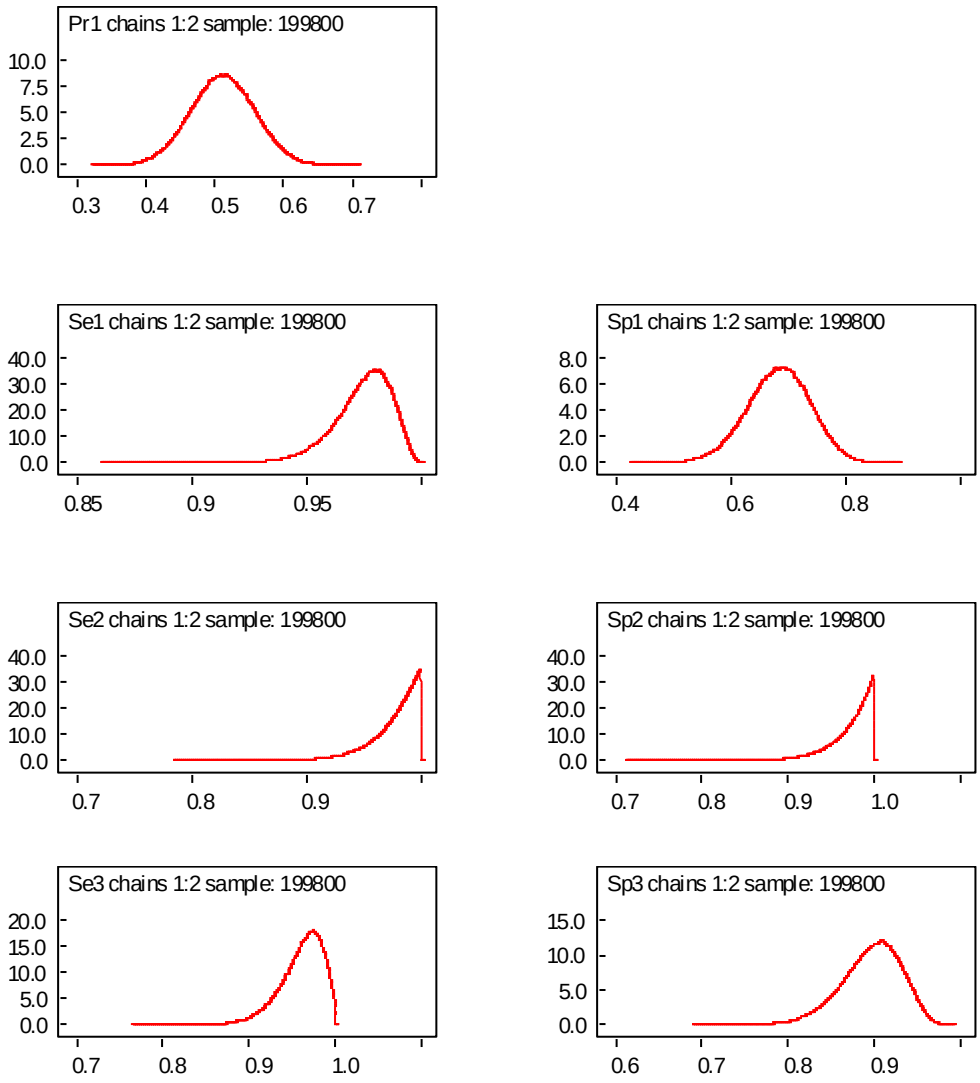
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.2.3: Kernel density estimate for the data set for the bovine animals.



Pr1 = Prevalence of disease in the population.

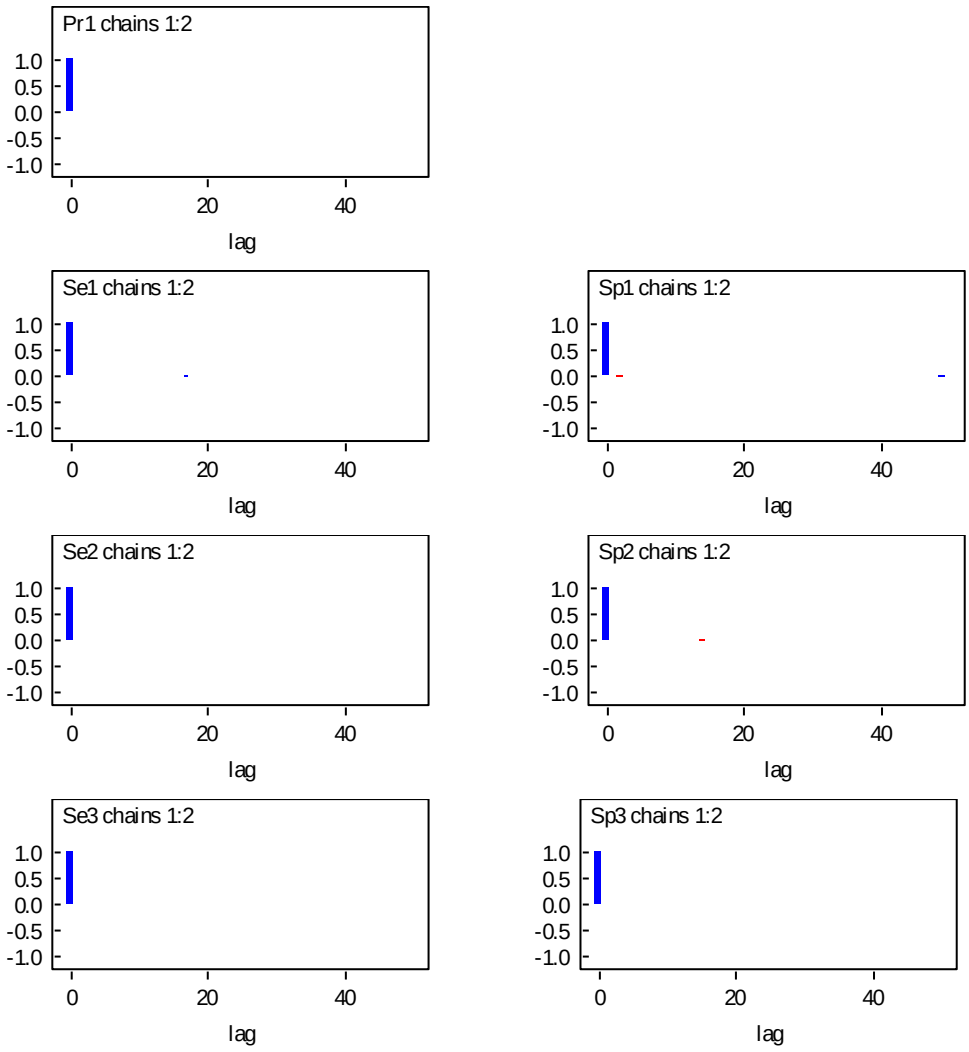
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.2.4: Autocorrelation plots for the data set for the bovine animals.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.3: Analysis of the data set for the 261 ovine animals.

Appendix 9.3.1: Posterior values obtained for each parameter for the data set for the ovine animals.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5676	0.03167	1.60E-04	0.5047	0.5679	0.6286	101	39800	0.57 [0.50, 0.63]
Se1	0.9749	0.00993	4.97E-05	0.9522	0.9761	0.9906	101	39800	0.98 [0.95, 0.99]
Se2	0.961	0.02073	1.06E-04	0.9148	0.9631	0.9949	101	39800	0.96 [0.91, 0.99]
Se3	0.9358	0.02049	1.05E-04	0.8906	0.9379	0.9706	101	39800	0.94 [0.89, 0.97]
Sp1	0.7108	0.04154	2.06E-04	0.6259	0.7118	0.7889	101	39800	0.71 [0.63, 0.79]
Sp2	0.9861	0.0138	7.05E-05	0.9486	0.9902	0.9996	101	39800	0.99 [0.95, 0.99]
Sp3	0.9169	0.02705	1.32E-04	0.8576	0.9194	0.9625	101	39800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5676	0.03177	1.13E-04	0.5048	0.5679	0.6291	101	79800	0.57 [0.50, 0.63]
Se1	0.9749	0.00988	3.58E-05	0.9523	0.9761	0.9906	101	79800	0.98 [0.95, 0.99]
Se2	0.961	0.02069	7.36E-05	0.9151	0.963	0.9949	101	79800	0.96 [0.92, 0.99]
Se3	0.9357	0.02058	7.16E-05	0.8903	0.9376	0.9705	101	79800	0.94 [0.89, 0.97]
Sp1	0.711	0.0417	1.44E-04	0.6258	0.7121	0.7899	101	79800	0.71 [0.63, 0.79]
Sp2	0.986	0.01388	5.26E-05	0.9484	0.9902	0.9996	101	79800	0.99 [0.95, 0.99]
Sp3	0.9168	0.02708	9.95E-05	0.8577	0.9192	0.9623	101	79800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5677	0.0318	9.28E-05	0.5048	0.5678	0.6291	101	119800	0.57 [0.50, 0.63]
Se1	0.9748	0.00992	2.78E-05	0.9522	0.9761	0.9906	101	119800	0.98 [0.95, 0.99]
Se2	0.961	0.02077	6.12E-05	0.9147	0.9631	0.995	101	119800	0.96 [0.91, 0.99]
Se3	0.9357	0.02063	6.00E-05	0.8902	0.9376	0.9705	101	119800	0.94 [0.89, 0.97]
Sp1	0.711	0.04175	1.21E-04	0.6261	0.7121	0.7898	101	119800	0.71 [0.63, 0.79]
Sp2	0.986	0.01382	3.97E-05	0.9487	0.9902	0.9996	101	119800	0.99 [0.95, 0.99]
Sp3	0.9168	0.02711	7.88E-05	0.8575	0.9192	0.9623	101	119800	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5676	0.03182	7.87E-05	0.5045	0.5678	0.6291	101	159800	0.57 [0.50, 0.63]
Se1	0.9748	0.00993	2.44E-05	0.9522	0.976	0.9906	101	159800	0.98 [0.95, 0.99]
Se2	0.961	0.02077	5.45E-05	0.9145	0.9631	0.9949	101	159800	0.96 [0.91, 0.99]
Se3	0.9357	0.02065	5.06E-05	0.8902	0.9376	0.9706	101	159800	0.94 [0.89, 0.97]
Sp1	0.711	0.04168	1.01E-04	0.6263	0.7121	0.7896	101	159800	0.71 [0.63, 0.79]
Sp2	0.9859	0.01384	3.59E-05	0.9486	0.9901	0.9996	101	159800	0.99 [0.95, 0.99]
Sp3	0.9168	0.02709	6.81E-05	0.8573	0.9193	0.9624	101	159800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5675	0.03185	7.46E-05	0.5045	0.5678	0.6291	101	199800	0.57 [0.50, 0.63]
Se1	0.9748	0.00993	2.24E-05	0.9522	0.976	0.9906	101	199800	0.98 [0.95, 0.99]
Se2	0.961	0.02077	4.76E-05	0.9145	0.9631	0.9949	101	199800	0.96 [0.91, 0.99]
Se3	0.9357	0.02066	4.58E-05	0.8901	0.9376	0.9705	101	199800	0.94 [0.89, 0.97]
Sp1	0.711	0.04171	9.25E-05	0.6262	0.7121	0.7895	101	199800	0.71 [0.63, 0.79]
Sp2	0.986	0.01383	3.21E-05	0.9487	0.9901	0.9996	101	199800	0.99 [0.95, 0.99]
Sp3	0.9168	0.02712	6.04E-05	0.8571	0.9192	0.9624	101	199800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5675	0.03186	7.46E-05	0.5045	0.5677	0.6292	20000	200002	0.57 [0.50, 0.63]
Se1	0.9748	0.00994	2.16E-05	0.9521	0.976	0.9905	20000	200002	0.98 [0.95, 0.99]
Se2	0.961	0.02074	4.80E-05	0.9145	0.9632	0.9949	20000	200002	0.96 [0.91, 0.99]
Se3	0.9357	0.02069	4.58E-05	0.8901	0.9375	0.9707	20000	200002	0.94 [0.89, 0.97]
Sp1	0.711	0.04176	9.35E-05	0.6263	0.7122	0.7896	20000	200002	0.71 [0.63, 0.79]
Sp2	0.9859	0.01387	3.20E-05	0.9487	0.9901	0.9996	20000	200002	0.99 [0.95, 0.99]
Sp3	0.9167	0.02714	6.17E-05	0.8569	0.9192	0.9624	20000	200002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5674	0.03186	8.16E-05	0.5044	0.5677	0.6291	40000	160002	0.57 [0.50, 0.63]
Se1	0.9747	0.00997	2.42E-05	0.952	0.976	0.9905	40000	160002	0.98 [0.95, 0.99]
Se2	0.961	0.02077	5.49E-05	0.9143	0.9632	0.9949	40000	160002	0.96 [0.91, 0.99]
Se3	0.9357	0.02069	5.04E-05	0.8901	0.9376	0.9707	40000	160002	0.94 [0.89, 0.97]
Sp1	0.7109	0.04173	1.04E-04	0.6264	0.7122	0.7893	40000	160002	0.71 [0.63, 0.79]
Sp2	0.9859	0.01385	3.42E-05	0.9487	0.9901	0.9996	40000	160002	0.99 [0.95, 0.99]
Sp3	0.9167	0.02715	7.05E-05	0.8568	0.9193	0.9625	40000	160002	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5674	0.03186	9.60E-05	0.5042	0.5677	0.6291	60000	120002	0.57 [0.50, 0.63]
Se1	0.9748	0.00996	2.71E-05	0.952	0.976	0.9905	60000	120002	0.98 [0.95, 0.99]
Se2	0.9611	0.02072	6.11E-05	0.9144	0.9632	0.9948	60000	120002	0.96 [0.91, 0.99]
Se3	0.9357	0.02068	5.86E-05	0.8901	0.9376	0.9708	60000	120002	0.94 [0.89, 0.97]
Sp1	0.7109	0.0417	1.18E-04	0.6263	0.7122	0.7892	60000	120002	0.71 [0.63, 0.79]
Sp2	0.9859	0.01389	4.22E-05	0.9486	0.9901	0.9996	60000	120002	0.99 [0.95, 0.99]
Sp3	0.9167	0.02714	8.49E-05	0.8567	0.9193	0.9625	60000	120002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5674	0.03185	1.19E-04	0.5045	0.5676	0.6292	80000	80002	0.57 [0.50, 0.63]
Se1	0.9748	0.00997	3.37E-05	0.952	0.9761	0.9905	80000	80002	0.98 [0.95, 0.99]
Se2	0.9611	0.02068	7.84E-05	0.9146	0.9632	0.9948	80000	80002	0.96 [0.91, 0.99]
Se3	0.9357	0.02067	7.22E-05	0.89	0.9376	0.9708	80000	80002	0.94 [0.89, 0.97]
Sp1	0.7109	0.04181	1.49E-04	0.6261	0.7123	0.7894	80000	80002	0.71 [0.63, 0.79]
Sp2	0.9859	0.01389	5.21E-05	0.9487	0.9901	0.9996	80000	80002	0.99 [0.95, 0.99]
Sp3	0.9167	0.0272	9.87E-05	0.8566	0.9193	0.9624	80000	80002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5674	0.03172	1.62E-04	0.5047	0.5675	0.6293	100000	40002	0.57 [0.50, 0.63]
Se1	0.9748	0.00999	4.61E-05	0.9517	0.976	0.9905	100000	40002	0.98 [0.95, 0.99]
Se2	0.9612	0.02062	9.99E-05	0.9147	0.9634	0.9947	100000	40002	0.96 [0.91, 0.99]
Se3	0.9357	0.02066	9.94E-05	0.8904	0.9375	0.971	100000	40002	0.94 [0.89, 0.97]
Sp1	0.7108	0.04179	2.09E-04	0.6264	0.7122	0.7893	100000	40002	0.71 [0.63, 0.79]
Sp2	0.9858	0.01401	7.58E-05	0.9482	0.9901	0.9996	100000	40002	0.99 [0.95, 0.99]
Sp3	0.9167	0.02715	1.39E-04	0.8566	0.9194	0.9624	100000	40002	0.92 [0.86, 0.96]

Pr1 = Prevalence of disease in the population.

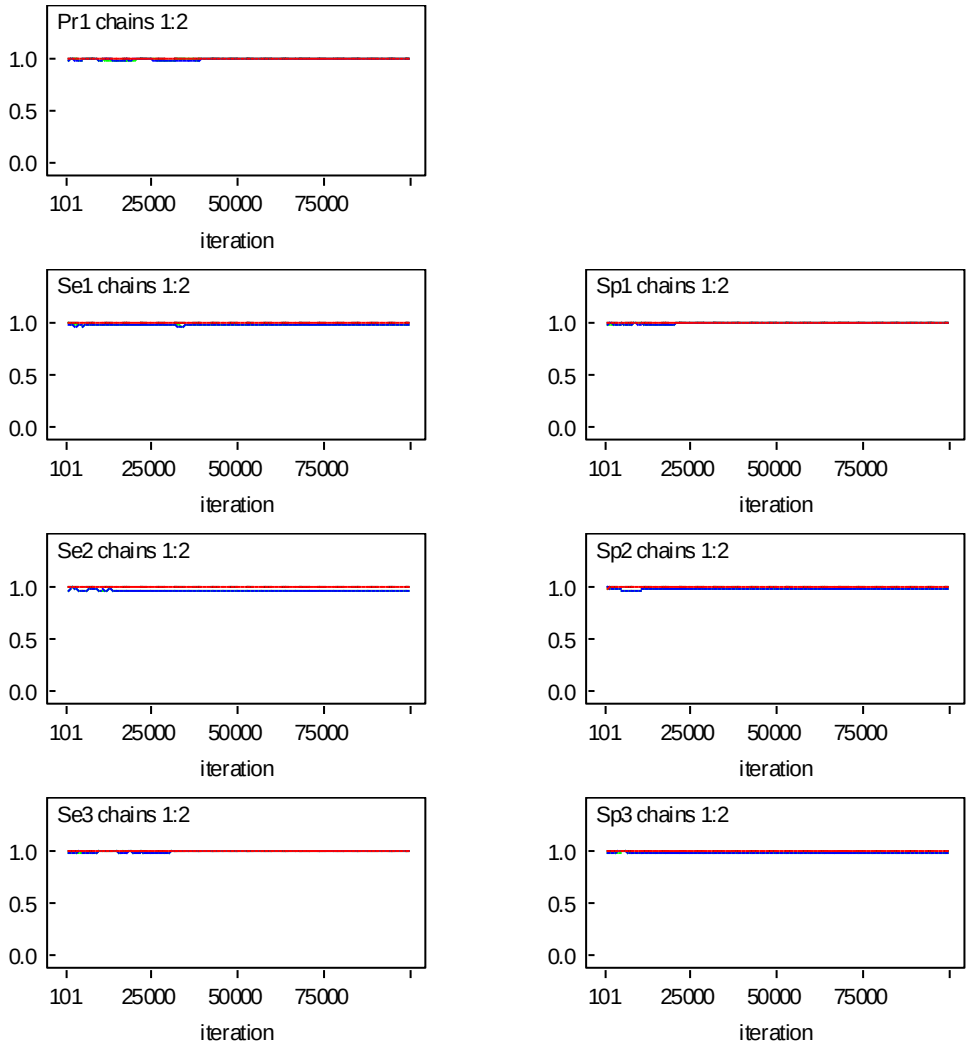
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.3.2: Plots of iterates for each variable to check for convergence for the data set for the ovine animals.



Pr1 = Prevalence of disease in the population.

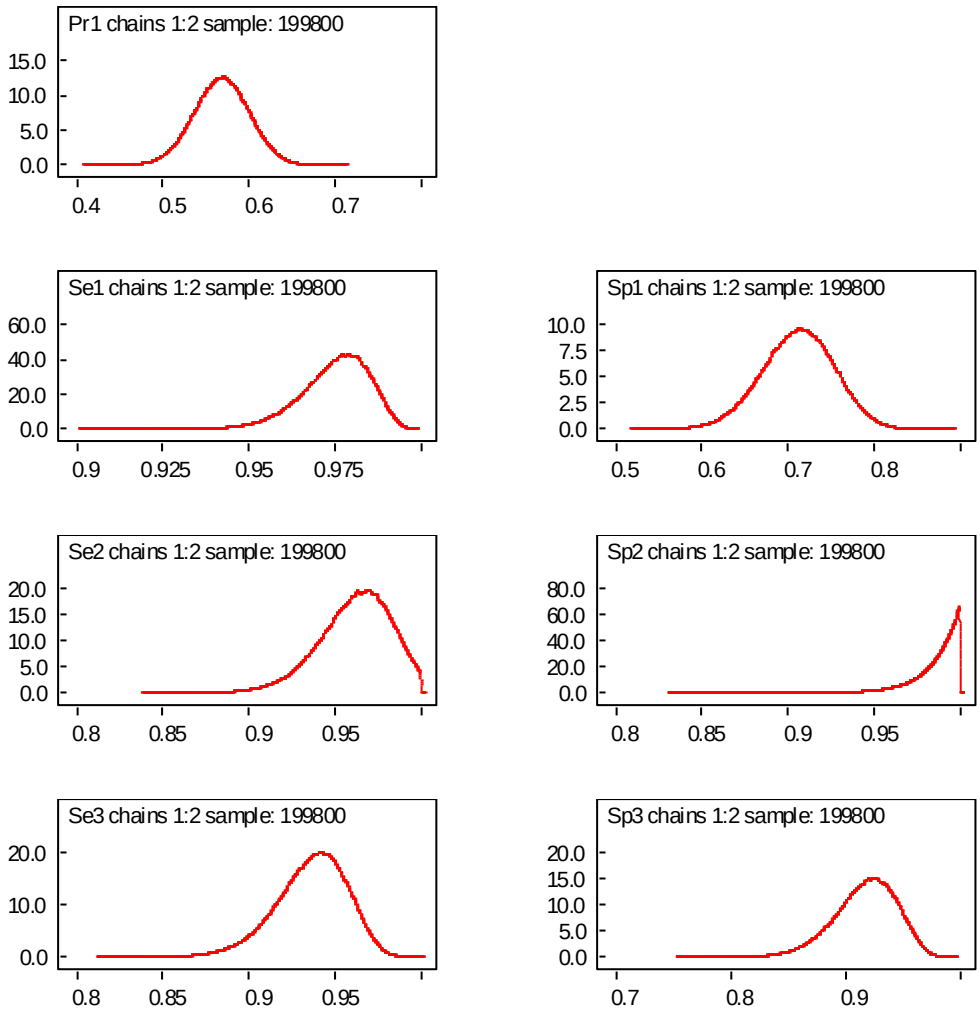
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.3.3: Kernel density estimate for the data set for the ovine animals.



Pr1 = Prevalence of disease in the population.

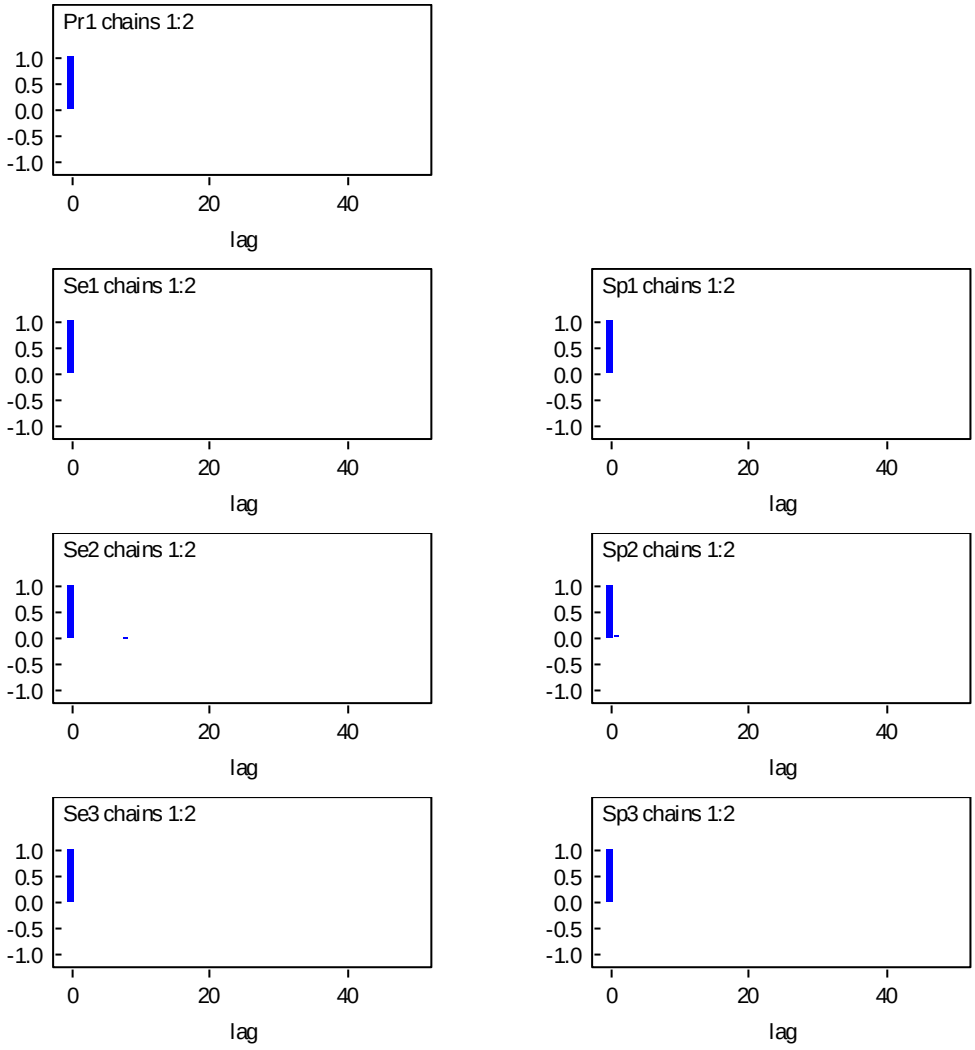
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.3.4: Autocorrelation plots for the data set for the ovine animals.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.4: Analysis of the data set for the 201 adult ovine and bovine animals.

Appendix 9.4.1: Posterior values obtained for each parameter for the data set for the adult ovine and bovine animals.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.496	0.03682	1.73E-04	0.4242	0.4961	0.5678	101	39800	0.50 [0.42, 0.57]
Se1	0.9738	0.01129	5.69E-05	0.9476	0.9754	0.9912	101	39800	0.98 [0.95, 0.99]
Se2	0.9477	0.02814	1.38E-04	0.8853	0.9508	0.9935	101	39800	0.95 [0.89, 0.99]
Se3	0.9354	0.02566	1.20E-04	0.8782	0.9382	0.9777	101	39800	0.94 [0.88, 0.98]
Sp1	0.695	0.04407	2.20E-04	0.6053	0.6962	0.7778	101	39800	0.70 [0.61, 0.78]
Sp2	0.984	0.01564	8.21E-05	0.9421	0.9887	0.9996	101	39800	0.99 [0.94, 0.99]
Sp3	0.9294	0.02629	1.37E-04	0.8704	0.9323	0.9723	101	39800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.496	0.03685	1.27E-04	0.4242	0.496	0.5681	101	79800	0.50 [0.42, 0.57]
Se1	0.9738	0.01124	3.86E-05	0.9478	0.9754	0.9911	101	79800	0.98 [0.95, 0.99]
Se2	0.9478	0.02815	9.74E-05	0.885	0.9508	0.9934	101	79800	0.95 [0.89, 0.99]
Se3	0.9353	0.02563	8.97E-05	0.878	0.938	0.9776	101	79800	0.94 [0.88, 0.98]
Sp1	0.6948	0.04415	1.58E-04	0.6052	0.696	0.7778	101	79800	0.70 [0.61, 0.78]
Sp2	0.984	0.0156	5.62E-05	0.9422	0.9888	0.9996	101	79800	0.99 [0.94, 0.99]
Sp3	0.9294	0.02632	9.46E-05	0.8704	0.9323	0.9723	101	79800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.496	0.03682	1.02E-04	0.4239	0.496	0.5681	101	119800	0.50 [0.42, 0.57]
Se1	0.9738	0.01122	3.23E-05	0.948	0.9753	0.9912	101	119800	0.98 [0.95, 0.99]
Se2	0.9477	0.02819	7.99E-05	0.8848	0.9507	0.9933	101	119800	0.95 [0.88, 0.99]
Se3	0.9354	0.02559	7.38E-05	0.878	0.9381	0.9777	101	119800	0.94 [0.88, 0.98]
Sp1	0.6948	0.04417	1.22E-04	0.6053	0.696	0.7778	101	119800	0.70 [0.61, 0.78]
Sp2	0.984	0.01558	4.59E-05	0.9422	0.9887	0.9996	101	119800	0.99 [0.94, 0.99]
Sp3	0.9295	0.02627	7.61E-05	0.8704	0.9323	0.9724	101	119800	0.93 [0.87, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.496	0.03681	8.84E-05	0.4239	0.4961	0.5683	101	159800	0.50 [0.42, 0.57]
Se1	0.9738	0.01123	2.70E-05	0.9478	0.9753	0.9912	101	159800	0.98 [0.95, 0.99]
Se2	0.9477	0.02816	6.59E-05	0.8848	0.9506	0.9933	101	159800	0.95 [0.88, 0.99]
Se3	0.9354	0.02557	6.44E-05	0.8782	0.9382	0.9777	101	159800	0.94 [0.88, 0.98]
Sp1	0.6949	0.04422	1.09E-04	0.6052	0.6961	0.778	101	159800	0.70 [0.61, 0.78]
Sp2	0.984	0.01556	4.05E-05	0.9422	0.9887	0.9996	101	159800	0.99 [0.94, 0.99]
Sp3	0.9295	0.02627	6.64E-05	0.8706	0.9324	0.9724	101	159800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.496	0.03679	7.99E-05	0.4239	0.496	0.5682	101	199800	0.50 [0.42, 0.57]
Se1	0.9738	0.01124	2.40E-05	0.9478	0.9753	0.9912	101	199800	0.98 [0.95, 0.99]
Se2	0.9476	0.02817	6.07E-05	0.8847	0.9506	0.9934	101	199800	0.95 [0.88, 0.99]
Se3	0.9354	0.02561	5.61E-05	0.878	0.9381	0.9777	101	199800	0.94 [0.88, 0.98]
Sp1	0.695	0.04419	9.26E-05	0.6051	0.6961	0.778	101	199800	0.70 [0.61, 0.78]
Sp2	0.9841	0.01555	3.58E-05	0.9424	0.9887	0.9996	101	199800	0.99 [0.94, 0.99]
Sp3	0.9295	0.02627	6.07E-05	0.8706	0.9323	0.9725	101	199800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.496	0.03678	8.16E-05	0.4239	0.496	0.5682	20000	200002	0.50 [0.42, 0.57]
Se1	0.9738	0.01124	2.43E-05	0.9478	0.9753	0.9912	20000	200002	0.98 [0.95, 0.99]
Se2	0.9476	0.02818	6.09E-05	0.8847	0.9506	0.9933	20000	200002	0.95 [0.88, 0.99]
Se3	0.9354	0.02558	5.70E-05	0.878	0.938	0.9776	20000	200002	0.94 [0.88, 0.98]
Sp1	0.695	0.04423	9.49E-05	0.6052	0.6961	0.7782	20000	200002	0.70 [0.61, 0.78]
Sp2	0.9841	0.01554	3.61E-05	0.9424	0.9888	0.9996	20000	200002	0.99 [0.94, 0.99]
Sp3	0.9295	0.02628	5.97E-05	0.8706	0.9322	0.9725	20000	200002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4961	0.03676	9.39E-05	0.4239	0.4961	0.5681	40000	160002	0.50 [0.42, 0.57]
Se1	0.9738	0.01125	2.76E-05	0.9478	0.9753	0.9912	40000	160002	0.98 [0.95, 0.99]
Se2	0.9476	0.02819	7.06E-05	0.8846	0.9506	0.9933	40000	160002	0.95 [0.88, 0.99]
Se3	0.9354	0.02557	6.23E-05	0.8781	0.9381	0.9777	40000	160002	0.94 [0.88, 0.98]
Sp1	0.695	0.04423	1.05E-04	0.6052	0.6962	0.7783	40000	160002	0.70 [0.61, 0.78]
Sp2	0.9841	0.01554	4.01E-05	0.9424	0.9887	0.9996	40000	160002	0.99 [0.94, 0.99]
Sp3	0.9295	0.02626	6.73E-05	0.8707	0.9322	0.9726	40000	160002	0.93 [0.87, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4961	0.03675	1.08E-04	0.4241	0.4961	0.5681	60000	120002	0.50 [0.42, 0.57]
Se1	0.9738	0.01127	3.26E-05	0.9477	0.9753	0.9912	60000	120002	0.98 [0.95, 0.99]
Se2	0.9476	0.02816	8.19E-05	0.8847	0.9506	0.9934	60000	120002	0.95 [0.88, 0.99]
Se3	0.9353	0.02559	7.42E-05	0.878	0.938	0.9775	60000	120002	0.94 [0.88, 0.98]
Sp1	0.6951	0.04424	1.26E-04	0.605	0.6963	0.7784	60000	120002	0.70 [0.61, 0.78]
Sp2	0.9841	0.01554	4.69E-05	0.9425	0.9887	0.9996	60000	120002	0.99 [0.94, 0.99]
Sp3	0.9295	0.02629	7.81E-05	0.8709	0.9322	0.9726	60000	120002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4961	0.03675	1.29E-04	0.4241	0.496	0.5677	80000	80002	0.50 [0.42, 0.57]
Se1	0.9738	0.01127	3.94E-05	0.9478	0.9753	0.9912	80000	80002	0.98 [0.95, 0.99]
Se2	0.9476	0.0282	1.01E-04	0.8847	0.9507	0.9934	80000	80002	0.95 [0.88, 0.99]
Se3	0.9352	0.02562	8.70E-05	0.8778	0.9378	0.9776	80000	80002	0.94 [0.88, 0.98]
Sp1	0.6951	0.04418	1.55E-04	0.6052	0.6963	0.7784	80000	80002	0.70 [0.61, 0.78]
Sp2	0.9841	0.01556	5.90E-05	0.9425	0.9888	0.9996	80000	80002	0.99 [0.94, 0.99]
Sp3	0.9293	0.0263	9.19E-05	0.8706	0.932	0.9726	80000	80002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4962	0.03678	1.79E-04	0.4243	0.4962	0.5675	100000	40002	0.50 [0.42, 0.57]
Se1	0.9738	0.01128	5.44E-05	0.9477	0.9753	0.9912	100000	40002	0.98 [0.95, 0.99]
Se2	0.9477	0.02819	1.36E-04	0.8849	0.9507	0.9932	100000	40002	0.95 [0.88, 0.99]
Se3	0.9353	0.02551	1.26E-04	0.8783	0.9378	0.9774	100000	40002	0.94 [0.88, 0.98]
Sp1	0.6951	0.04426	2.36E-04	0.6053	0.6962	0.7785	100000	40002	0.70 [0.61, 0.78]
Sp2	0.984	0.01559	8.31E-05	0.9423	0.9888	0.9996	100000	40002	0.99 [0.94, 0.99]
Sp3	0.9293	0.02634	1.31E-04	0.8705	0.932	0.9725	100000	40002	0.93 [0.87, 0.97]

Pr1 = Prevalence of disease in the population.

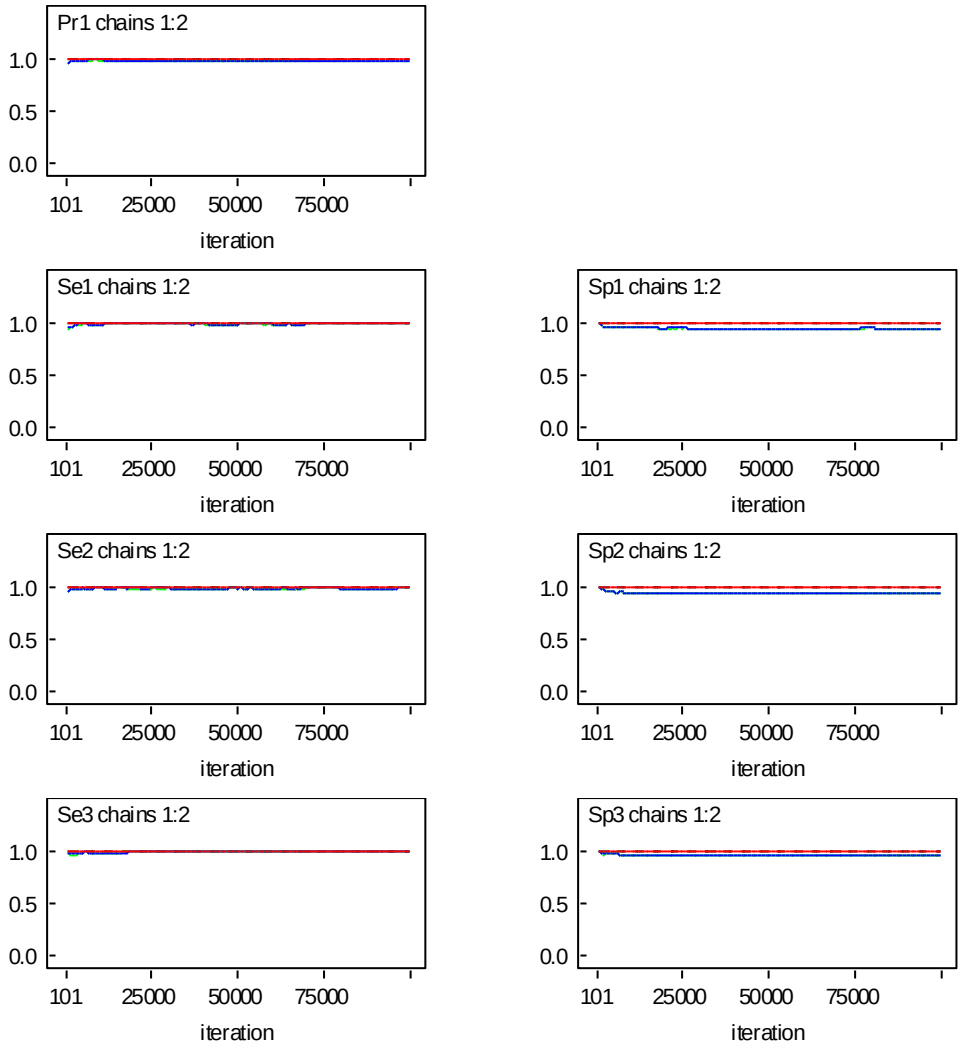
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.4.2: Plots of iterates for each variable to check for convergence for the data set for the adult ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

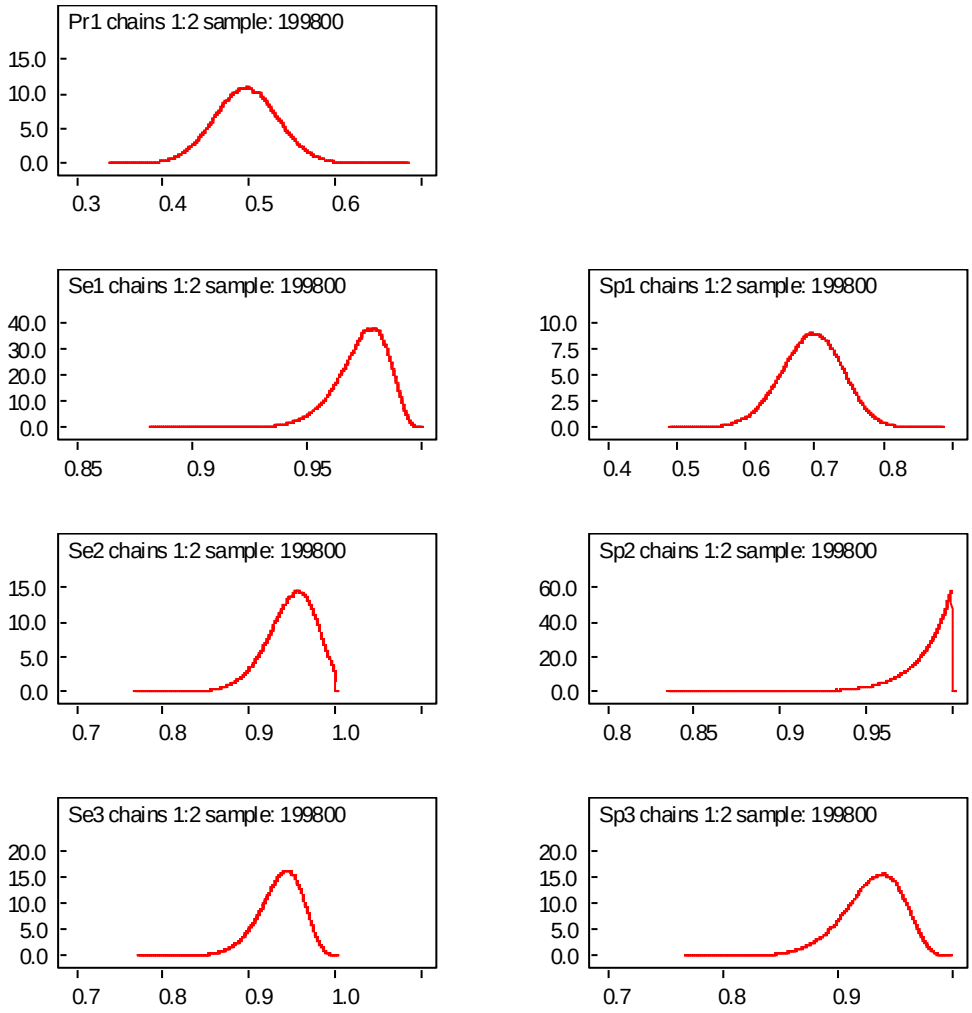
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.4.3: Kernel density estimate for the data set for the data set for the adult ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

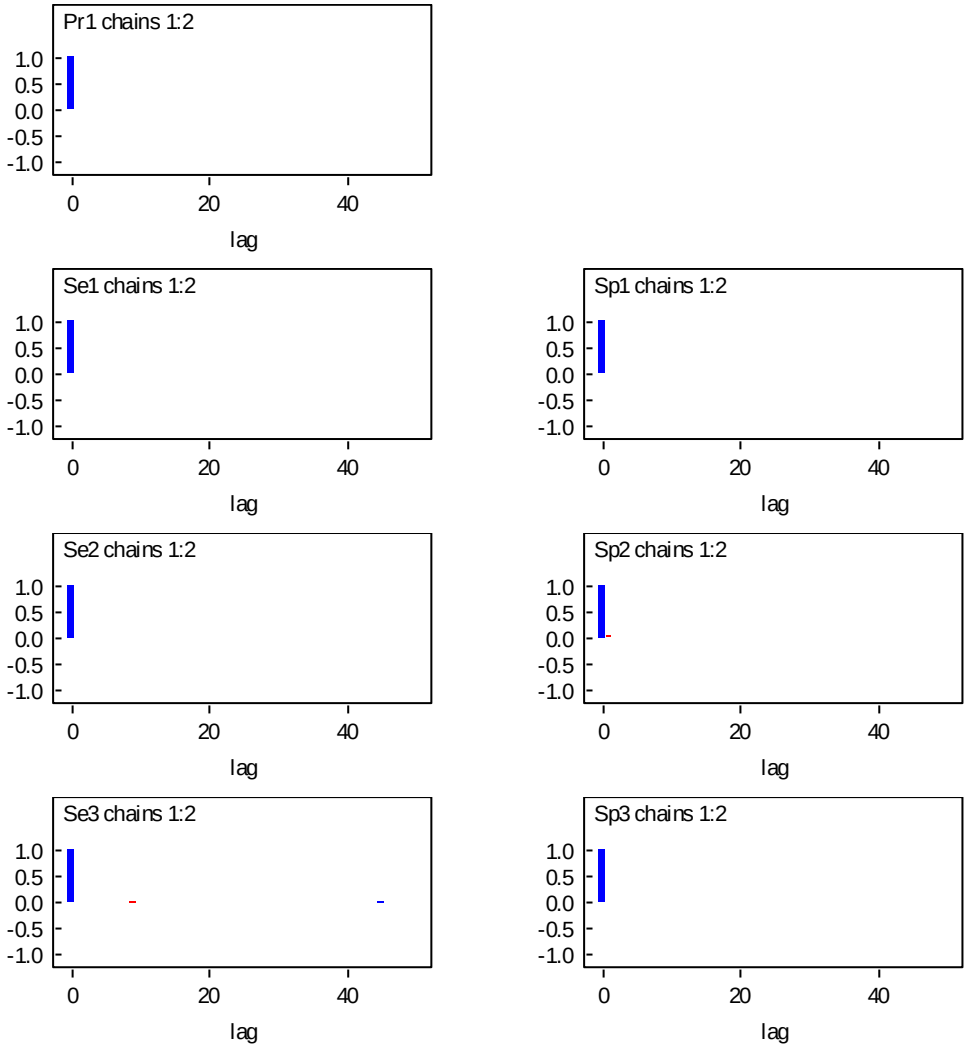
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.4.4: Autocorrelation plots for the data set for the adult ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.5: Analysis of the data set for the 85 neonatal ovine and bovine animals.

Appendix 9.5.1: Posterior values obtained for each parameter for the data set for the neonatal ovine and bovine animals.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5684	0.05382	2.76E-04	0.4606	0.5693	0.6708	101	39800	0.57 [0.46, 0.67]
Se1	0.9723	0.01325	6.33E-05	0.9417	0.9742	0.9924	101	39800	0.97 [0.94, 0.99]
Se2	0.9784	0.02128	1.07E-04	0.9221	0.9848	0.9994	101	39800	0.98 [0.92, 0.99]
Se3	0.9516	0.03066	1.49E-04	0.8775	0.9569	0.9947	101	39800	0.96 [0.88, 0.99]
Sp1	0.6588	0.06587	3.08E-04	0.523	0.6613	0.7808	101	39800	0.66 [0.52, 0.78]
Sp2	0.9581	0.03812	1.90E-04	0.8582	0.9691	0.9988	101	39800	0.97 [0.86, 0.99]
Sp3	0.9196	0.03603	1.84E-04	0.8366	0.9244	0.975	101	39800	0.92 [0.84, 0.98]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5683	0.05386	1.98E-04	0.461	0.5692	0.6711	101	79800	0.57 [0.46, 0.67]
Se1	0.9724	0.01331	4.52E-05	0.9412	0.9743	0.9924	101	79800	0.97 [0.94, 0.99]
Se2	0.9784	0.0212	7.05E-05	0.9218	0.9848	0.9994	101	79800	0.98 [0.92, 0.99]
Se3	0.9518	0.03069	1.07E-04	0.8781	0.9572	0.9949	101	79800	0.96 [0.88, 0.99]
Sp1	0.6587	0.06598	2.24E-04	0.523	0.661	0.7811	101	79800	0.66 [0.52, 0.78]
Sp2	0.9581	0.03804	1.32E-04	0.8591	0.9689	0.9988	101	79800	0.97 [0.86, 0.99]
Sp3	0.9196	0.03598	1.23E-04	0.8369	0.9244	0.9749	101	79800	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5684	0.05383	1.53E-04	0.461	0.5694	0.6717	101	119800	0.57 [0.46, 0.67]
Se1	0.9723	0.0133	3.75E-05	0.9411	0.9743	0.9923	101	119800	0.97 [0.94, 0.99]
Se2	0.9784	0.02112	6.11E-05	0.9221	0.9847	0.9994	101	119800	0.98 [0.92, 0.99]
Se3	0.9517	0.03077	9.04E-05	0.8778	0.9571	0.9949	101	119800	0.96 [0.88, 0.99]
Sp1	0.6587	0.06602	1.87E-04	0.523	0.661	0.781	101	119800	0.66 [0.52, 0.78]
Sp2	0.9582	0.03793	1.11E-04	0.8595	0.969	0.9988	101	119800	0.97 [0.86, 0.99]
Sp3	0.9194	0.036	1.06E-04	0.8365	0.9243	0.9748	101	119800	0.92 [0.84, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5682	0.05381	1.41E-04	0.4609	0.5689	0.6716	101	159800	0.57 [0.46, 0.67]
Se1	0.9723	0.0133	3.34E-05	0.9412	0.9743	0.9923	101	159800	0.97 [0.94, 0.99]
Se2	0.9785	0.02111	5.59E-05	0.9219	0.9848	0.9994	101	159800	0.98 [0.92, 0.99]
Se3	0.9517	0.03077	7.93E-05	0.8779	0.9572	0.995	101	159800	0.96 [0.88, 0.99]
Sp1	0.6587	0.06598	1.59E-04	0.5232	0.6609	0.781	101	159800	0.66 [0.52, 0.78]
Sp2	0.9581	0.03801	9.68E-05	0.8593	0.9689	0.9988	101	159800	0.97 [0.86, 0.99]
Sp3	0.9193	0.03602	9.21E-05	0.8361	0.9242	0.9747	101	159800	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5683	0.0538	1.22E-04	0.4611	0.569	0.6719	101	199800	0.57 [0.46, 0.67]
Se1	0.9723	0.01332	3.09E-05	0.9411	0.9743	0.9923	101	199800	0.97 [0.94, 0.99]
Se2	0.9785	0.0211	4.67E-05	0.922	0.9848	0.9994	101	199800	0.98 [0.92, 0.99]
Se3	0.9518	0.03073	7.08E-05	0.8781	0.9572	0.995	101	199800	0.96 [0.88, 0.99]
Sp1	0.6587	0.06594	1.41E-04	0.5236	0.6608	0.7812	101	199800	0.66 [0.52, 0.78]
Sp2	0.958	0.03799	9.10E-05	0.8594	0.9688	0.9988	101	199800	0.97 [0.86, 0.99]
Sp3	0.9194	0.03599	7.97E-05	0.8362	0.9242	0.9747	101	199800	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5683	0.05378	1.24E-04	0.4615	0.5689	0.6723	20000	200002	0.57 [0.46, 0.67]
Se1	0.9723	0.01333	3.04E-05	0.941	0.9743	0.9923	20000	200002	0.97 [0.94, 0.99]
Se2	0.9785	0.0211	4.76E-05	0.9219	0.9849	0.9994	20000	200002	0.98 [0.92, 0.99]
Se3	0.9518	0.0308	7.08E-05	0.878	0.9572	0.9951	20000	200002	0.96 [0.88, 0.99]
Sp1	0.6587	0.06601	1.47E-04	0.5239	0.6607	0.7814	20000	200002	0.66 [0.52, 0.78]
Sp2	0.958	0.03796	9.17E-05	0.8597	0.9688	0.9988	20000	200002	0.97 [0.86, 0.99]
Sp3	0.9193	0.03603	7.95E-05	0.836	0.9242	0.9747	20000	200002	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5684	0.05375	1.42E-04	0.4616	0.5689	0.6725	40000	160002	0.57 [0.46, 0.67]
Se1	0.9723	0.01332	3.47E-05	0.941	0.9743	0.9923	40000	160002	0.97 [0.94, 0.99]
Se2	0.9785	0.02109	5.31E-05	0.9219	0.9849	0.9994	40000	160002	0.98 [0.92, 0.99]
Se3	0.9517	0.03082	7.86E-05	0.8778	0.9571	0.9951	40000	160002	0.96 [0.88, 0.99]
Sp1	0.6587	0.06599	1.71E-04	0.5241	0.6607	0.7815	40000	160002	0.66 [0.52, 0.78]
Sp2	0.958	0.03796	1.01E-04	0.8596	0.9688	0.9988	40000	160002	0.97 [0.86, 0.99]
Sp3	0.9193	0.03605	8.84E-05	0.8357	0.9242	0.9746	40000	160002	0.92 [0.84, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5683	0.05374	1.61E-04	0.4617	0.5686	0.6725	60000	120002	0.57 [0.46, 0.67]
Se1	0.9723	0.01333	4.09E-05	0.941	0.9743	0.9923	60000	120002	0.97 [0.94, 0.99]
Se2	0.9786	0.02113	6.19E-05	0.9217	0.985	0.9994	60000	120002	0.99 [0.92, 0.99]
Se3	0.9518	0.03078	9.45E-05	0.8781	0.9572	0.9951	60000	120002	0.96 [0.88, 0.99]
Sp1	0.6587	0.06596	1.85E-04	0.5246	0.6606	0.7817	60000	120002	0.66 [0.52, 0.78]
Sp2	0.9579	0.03805	1.11E-04	0.8594	0.9687	0.9988	60000	120002	0.97 [0.86, 0.99]
Sp3	0.9193	0.03605	1.05E-04	0.8358	0.9243	0.9747	60000	120002	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5687	0.05374	1.87E-04	0.4624	0.5691	0.6729	80000	80002	0.57 [0.46, 0.67]
Se1	0.9723	0.01335	5.03E-05	0.9408	0.9743	0.9923	80000	80002	0.97 [0.94, 0.99]
Se2	0.9785	0.02115	7.34E-05	0.9219	0.9849	0.9994	80000	80002	0.98 [0.92, 0.99]
Se3	0.9518	0.03079	1.13E-04	0.8779	0.9572	0.9951	80000	80002	0.96 [0.88, 0.99]
Sp1	0.6587	0.06602	2.34E-04	0.5252	0.6604	0.7819	80000	80002	0.66 [0.53, 0.78]
Sp2	0.958	0.03794	1.42E-04	0.86	0.9688	0.9988	80000	80002	0.97 [0.86, 0.99]
Sp3	0.9195	0.03603	1.24E-04	0.8361	0.9244	0.9748	80000	80002	0.92 [0.84, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5687	0.05372	2.77E-04	0.463	0.569	0.6727	100000	40002	0.57 [0.46, 0.67]
Se1	0.9723	0.01333	6.76E-05	0.941	0.9744	0.9923	100000	40002	0.97 [0.94, 0.99]
Se2	0.9786	0.02124	1.09E-04	0.9216	0.985	0.9994	100000	40002	0.99 [0.92, 0.99]
Se3	0.9516	0.031	1.61E-04	0.877	0.957	0.9951	100000	40002	0.96 [0.88, 0.99]
Sp1	0.6589	0.06622	3.41E-04	0.5247	0.6607	0.7817	100000	40002	0.66 [0.52, 0.78]
Sp2	0.9582	0.03801	1.97E-04	0.8595	0.969	0.9989	100000	40002	0.97 [0.86, 0.99]
Sp3	0.9195	0.03623	1.81E-04	0.8355	0.9246	0.9747	100000	40002	0.92 [0.84, 0.97]

Pr1 = Prevalence of disease in the population.

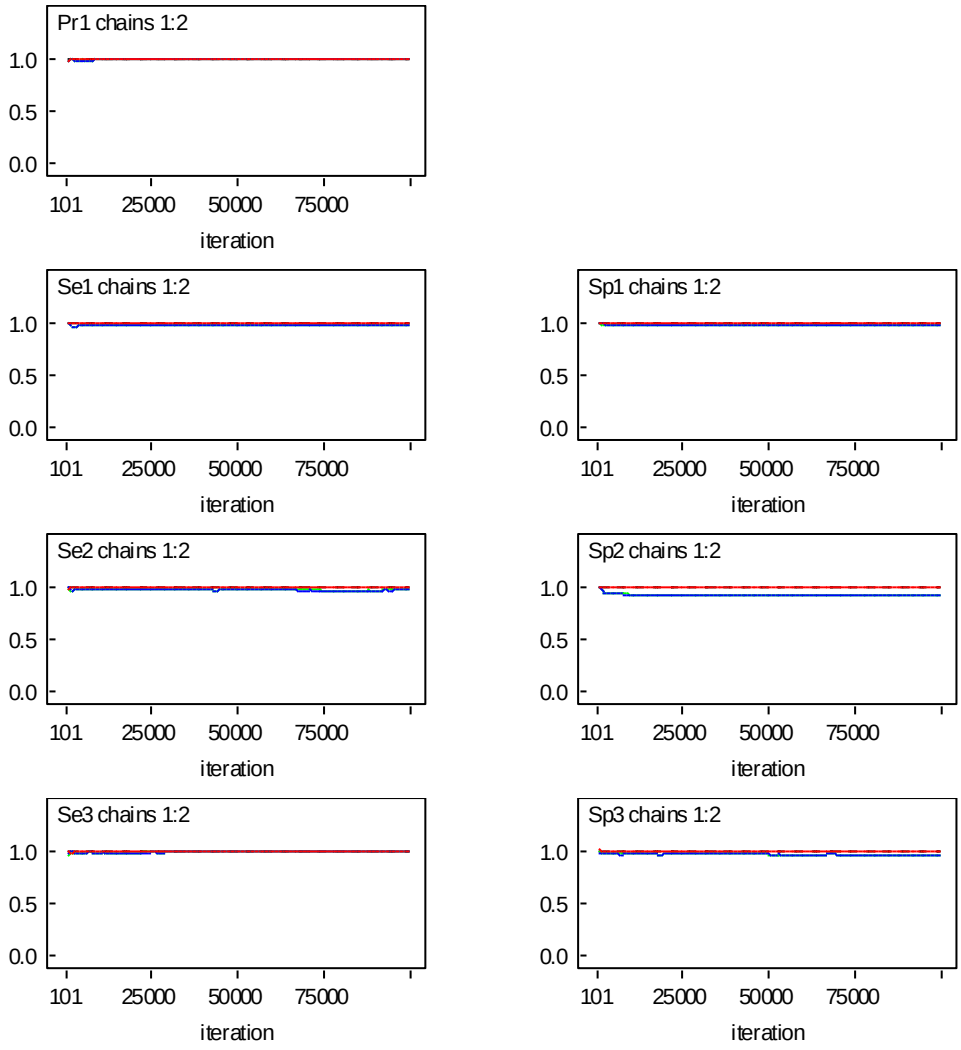
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.5.2: Plots of iterates for each variable to check for convergence for the data set for the neonatal ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

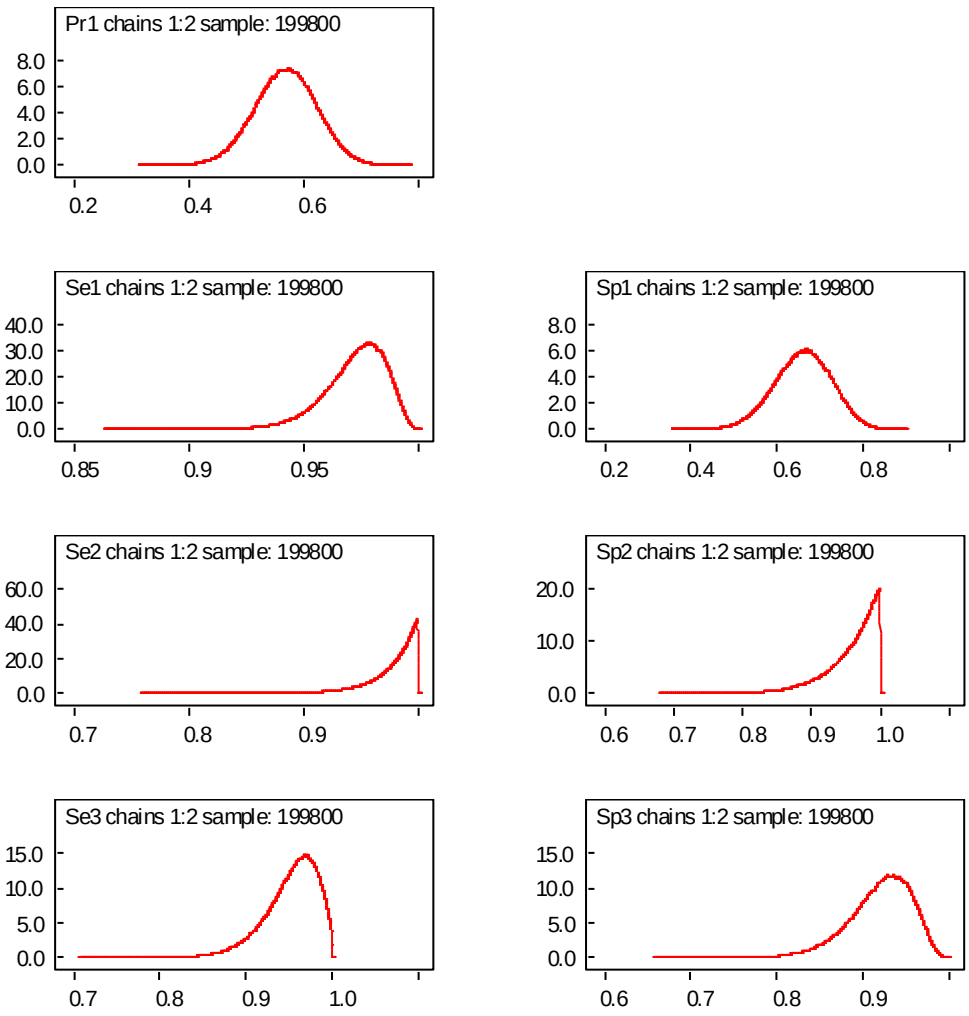
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.5.3: Kernel density estimate for the data set for the neonatal ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

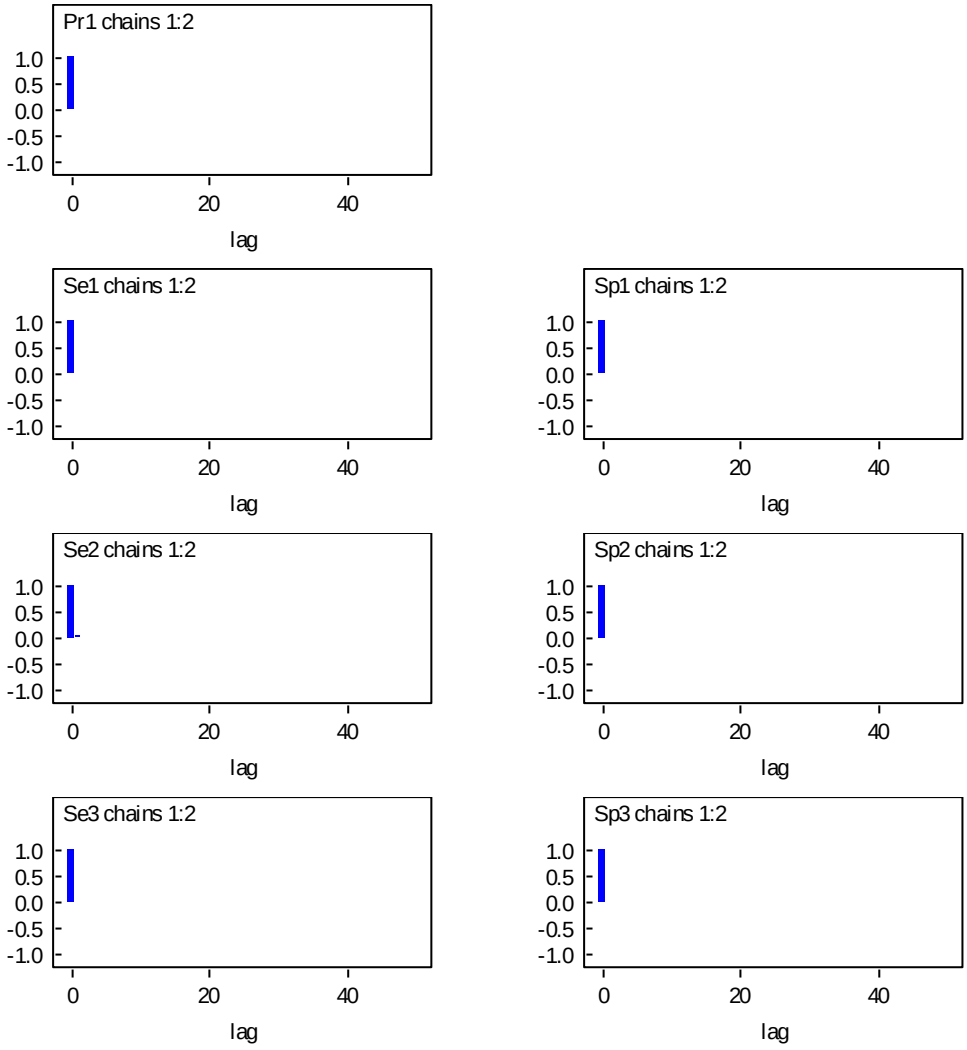
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.5.4: Autocorrelation plots for the data set for the neonatal ovine and bovine animals.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.6: Analysis of the data set for the 94 bovine and ovine foetuses.

Appendix 9.6.1: Posterior values obtained for each parameter for the data set for the bovine and ovine foetuses.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6446	0.05044	2.46E-04	0.5433	0.6455	0.7404	101	39800	0.65 [0.54, 0.74]
Se1	0.9801	0.01112	5.54E-05	0.9533	0.9821	0.9958	101	39800	0.98 [0.95, 0.99]
Se2	0.9629	0.0279	1.39E-04	0.8944	0.9687	0.9982	101	39800	0.97 [0.89, 0.99]
Se3	0.9424	0.0295	1.56E-04	0.8737	0.9466	0.9875	101	39800	0.95 [0.87, 0.99]
Sp1	0.787	0.061	3.12E-04	0.6561	0.7909	0.8945	101	39800	0.79 [0.66, 0.89]
Sp2	0.9577	0.03928	2.05E-04	0.8546	0.9691	0.9988	101	39800	0.97 [0.85, 0.99]
Sp3	0.8266	0.05229	2.67E-04	0.7145	0.8305	0.9167	101	39800	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.6445	0.05038	1.80E-04	0.5431	0.6456	0.7403	101	79800	0.65 [0.54, 0.74]
Se1	0.9801	0.01106	4.04E-05	0.9534	0.982	0.9957	101	79800	0.98 [0.95, 0.99]
Se2	0.9628	0.02801	1.00E-04	0.8938	0.9687	0.9982	101	79800	0.97 [0.89, 0.99]
Se3	0.9423	0.02964	1.09E-04	0.8734	0.9465	0.9875	101	79800	0.95 [0.87, 0.99]
Sp1	0.787	0.06093	2.07E-04	0.6575	0.7907	0.8947	101	79800	0.79 [0.66, 0.89]
Sp2	0.9578	0.03909	1.41E-04	0.8549	0.9692	0.9988	101	79800	0.97 [0.85, 0.99]
Sp3	0.8264	0.05249	1.90E-04	0.7137	0.8305	0.9172	101	79800	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.6445	0.05044	1.50E-04	0.5429	0.6455	0.7403	101	119800	0.65 [0.54, 0.74]
Se1	0.98	0.01105	3.23E-05	0.9534	0.982	0.9957	101	119800	0.98 [0.95, 0.99]
Se2	0.9628	0.02795	8.00E-05	0.8939	0.9686	0.9982	101	119800	0.97 [0.89, 0.99]
Se3	0.9423	0.02961	8.80E-05	0.8733	0.9466	0.9873	101	119800	0.95 [0.87, 0.99]
Sp1	0.787	0.06096	1.66E-04	0.6575	0.7907	0.8943	101	119800	0.79 [0.66, 0.89]
Sp2	0.9579	0.039	1.09E-04	0.8554	0.9691	0.9988	101	119800	0.97 [0.86, 0.99]
Sp3	0.8264	0.05246	1.47E-04	0.7135	0.8304	0.9172	101	119800	0.83 [0.71, 0.92]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6444	0.05048	1.26E-04	0.5427	0.6455	0.7402	101	159800	0.65 [0.54, 0.74]
Se1	0.98	0.01104	2.86E-05	0.9534	0.982	0.9957	101	159800	0.98 [0.95, 0.99]
Se2	0.9628	0.02797	7.04E-05	0.8939	0.9686	0.9982	101	159800	0.97 [0.89, 0.99]
Se3	0.9422	0.02968	7.68E-05	0.873	0.9466	0.9873	101	159800	0.95 [0.87, 0.99]
Sp1	0.7871	0.06098	1.53E-04	0.6576	0.7909	0.8942	101	159800	0.79 [0.66, 0.89]
Sp2	0.9578	0.03914	9.63E-05	0.8546	0.9692	0.9988	101	159800	0.97 [0.85, 0.99]
Sp3	0.8265	0.05254	1.34E-04	0.7129	0.8305	0.9172	101	159800	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6444	0.05042	1.12E-04	0.5427	0.6455	0.7401	101	199800	0.65 [0.54, 0.74]
Se1	0.98	0.01104	2.49E-05	0.9535	0.982	0.9957	101	199800	0.98 [0.95, 0.99]
Se2	0.9628	0.02797	6.03E-05	0.8939	0.9686	0.9982	101	199800	0.97 [0.89, 0.99]
Se3	0.9423	0.02967	6.72E-05	0.8729	0.9466	0.9873	101	199800	0.95 [0.87, 0.99]
Sp1	0.7871	0.06111	1.39E-04	0.6571	0.7909	0.8943	101	199800	0.79 [0.66, 0.89]
Sp2	0.9578	0.03918	8.95E-05	0.8545	0.9692	0.9988	101	199800	0.97 [0.85, 0.99]
Sp3	0.8265	0.05253	1.17E-04	0.713	0.8305	0.9171	101	199800	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6443	0.05044	1.18E-04	0.5425	0.6454	0.7398	20000	200002	0.65 [0.54, 0.74]
Se1	0.98	0.011	2.43E-05	0.9535	0.9819	0.9957	20000	200002	0.98 [0.95, 0.99]
Se2	0.9627	0.02803	6.16E-05	0.8938	0.9686	0.9982	20000	200002	0.97 [0.89, 0.99]
Se3	0.9423	0.02972	6.51E-05	0.8727	0.9466	0.9873	20000	200002	0.95 [0.87, 0.99]
Sp1	0.7871	0.06115	1.37E-04	0.6572	0.7909	0.8946	20000	200002	0.79 [0.66, 0.89]
Sp2	0.9577	0.03928	8.77E-05	0.8537	0.9691	0.9988	20000	200002	0.97 [0.85, 0.99]
Sp3	0.8264	0.05254	1.19E-04	0.7126	0.8304	0.917	20000	200002	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6442	0.05047	1.28E-04	0.5424	0.6454	0.7397	40000	160002	0.65 [0.54, 0.74]
Se1	0.98	0.011	2.62E-05	0.9535	0.9819	0.9958	40000	160002	0.98 [0.95, 0.99]
Se2	0.9627	0.02801	6.93E-05	0.8938	0.9686	0.9982	40000	160002	0.97 [0.89, 0.99]
Se3	0.9423	0.02971	6.93E-05	0.8726	0.9467	0.9872	40000	160002	0.95 [0.87, 0.99]
Sp1	0.7871	0.06122	1.55E-04	0.6567	0.791	0.8944	40000	160002	0.79 [0.66, 0.89]
Sp2	0.9577	0.03937	9.84E-05	0.8533	0.9691	0.9988	40000	160002	0.97 [0.85, 0.99]
Sp3	0.8264	0.05251	1.23E-04	0.7125	0.8304	0.9168	40000	160002	0.83 [0.71, 0.92]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6442	0.05044	1.49E-04	0.5424	0.6454	0.7393	60000	120002	0.65 [0.54, 0.74]
Se1	0.9801	0.01099	3.15E-05	0.9536	0.982	0.9958	60000	120002	0.98 [0.95, 0.99]
Se2	0.9627	0.02807	8.04E-05	0.8937	0.9686	0.9982	60000	120002	0.97 [0.89, 0.99]
Se3	0.9423	0.02976	8.65E-05	0.8724	0.9467	0.9873	60000	120002	0.95 [0.87, 0.99]
Sp1	0.7871	0.06129	1.80E-04	0.6562	0.7911	0.8948	60000	120002	0.79 [0.66, 0.89]
Sp2	0.9576	0.03956	1.14E-04	0.8524	0.9692	0.9988	60000	120002	0.97 [0.85, 0.99]
Sp3	0.8264	0.05254	1.49E-04	0.7124	0.8305	0.9168	60000	120002	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6442	0.05035	1.90E-04	0.5426	0.6453	0.7392	80000	80002	0.65 [0.54, 0.74]
Se1	0.9801	0.01098	3.77E-05	0.9536	0.982	0.9958	80000	80002	0.98 [0.95, 0.99]
Se2	0.9627	0.0281	1.00E-04	0.8937	0.9686	0.9982	80000	80002	0.97 [0.89, 0.99]
Se3	0.9423	0.02971	1.05E-04	0.8727	0.9467	0.9873	80000	80002	0.95 [0.87, 0.99]
Sp1	0.787	0.06142	2.21E-04	0.6556	0.791	0.8952	80000	80002	0.79 [0.66, 0.90]
Sp2	0.9575	0.03955	1.44E-04	0.8527	0.969	0.9988	80000	80002	0.97 [0.85, 0.99]
Sp3	0.8262	0.05243	1.88E-04	0.7129	0.8303	0.9166	80000	80002	0.83 [0.71, 0.92]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6441	0.05051	2.77E-04	0.5421	0.6453	0.7393	100000	40002	0.65 [0.54, 0.74]
Se1	0.9801	0.01094	5.64E-05	0.9536	0.9819	0.9958	100000	40002	0.98 [0.95, 0.99]
Se2	0.9626	0.02819	1.39E-04	0.8932	0.9685	0.9982	100000	40002	0.97 [0.89, 0.99]
Se3	0.9424	0.02978	1.42E-04	0.8728	0.9468	0.9875	100000	40002	0.95 [0.87, 0.99]
Sp1	0.7871	0.06122	2.99E-04	0.6565	0.791	0.8955	100000	40002	0.79 [0.66, 0.90]
Sp2	0.9574	0.03975	2.04E-04	0.8515	0.969	0.9988	100000	40002	0.97 [0.85, 0.99]
Sp3	0.826	0.05234	2.74E-04	0.7127	0.8299	0.9164	100000	40002	0.83 [0.71, 0.92]

Pr1 = Prevalence of disease in the population.

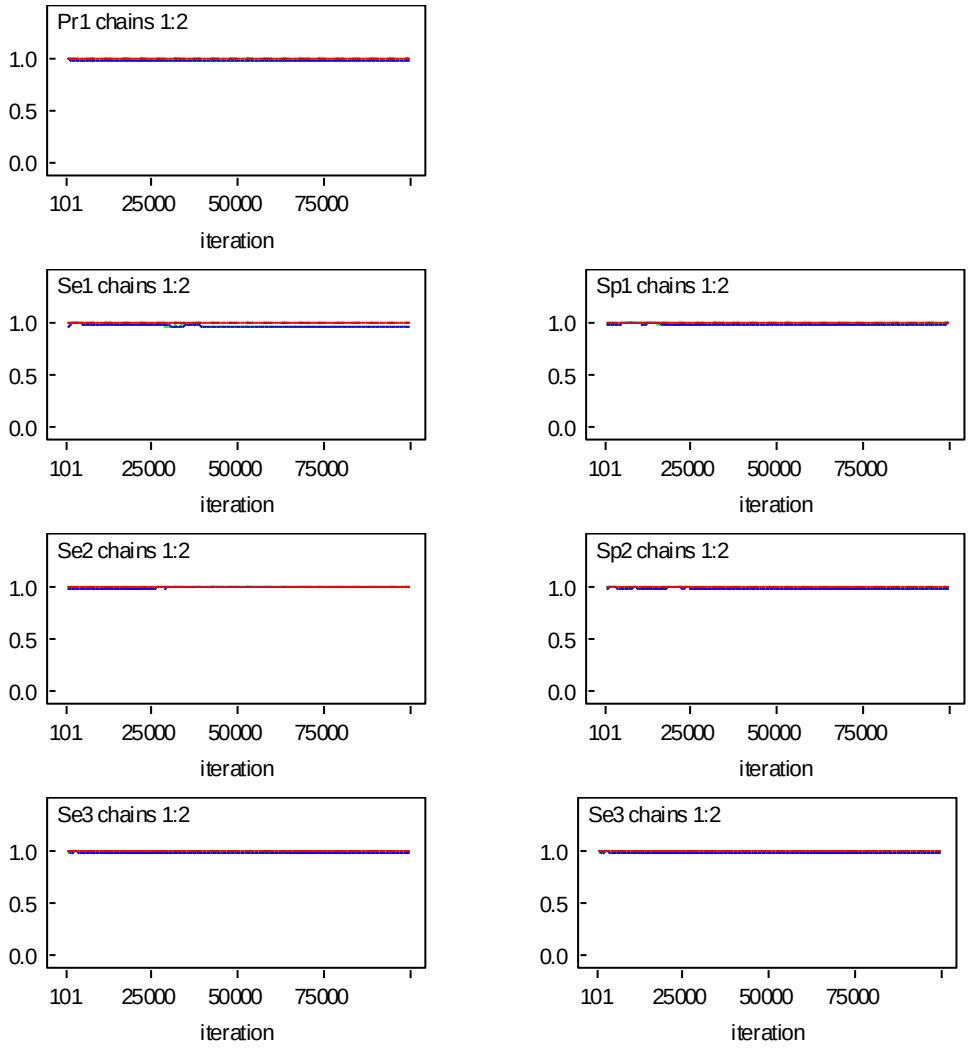
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.6.2: Plots of iterates for each variable to check for convergence for the data set for the bovine and ovine foetuses.



Pr1 = Prevalence of disease in the population.

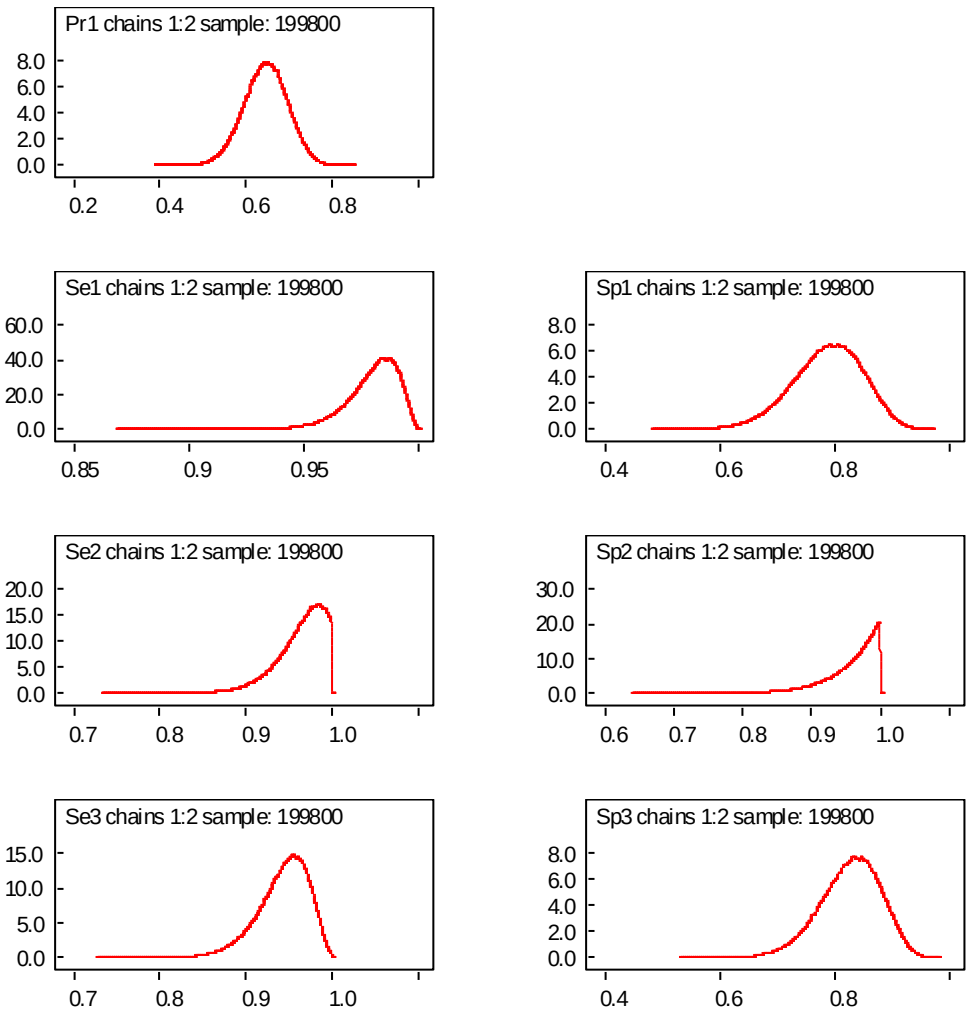
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.6.3: Kernel density estimate for the data set for the bovine and ovine foetuses.



Pr1 = Prevalence of disease in the population.

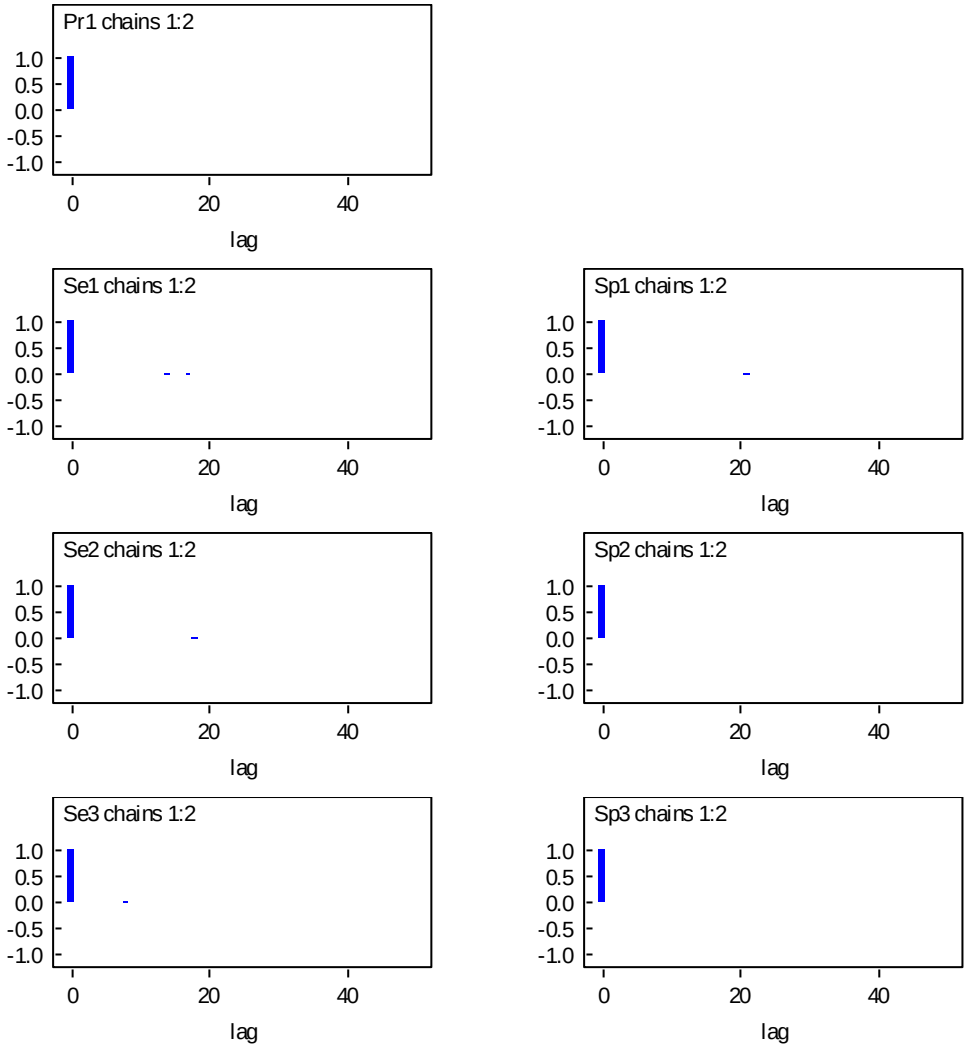
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.6.4: Autocorrelation plots for the data set for the bovine and ovine foetuses.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.7: Analysis of the data set for the 243 mildly autolysed cases.

Appendix 9.7.1: Posterior values obtained for each parameter for the data set for the mildly autolysed cases.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5611	0.03214	1.60E-04	0.4976	0.5613	0.6236	101	39800	0.56 [0.50, 0.62]
Se1	0.9779	0.00945	4.82E-05	0.9561	0.9793	0.9926	101	39800	0.98 [0.96, 0.99]
Se2	0.9859	0.0119	5.67E-05	0.9554	0.9888	0.9995	101	39800	0.99 [0.96, 0.99]
Se3	0.9527	0.01831	8.75E-05	0.9109	0.9548	0.9822	101	39800	0.95 [0.91, 0.98]
Sp1	0.7641	0.03895	1.89E-04	0.6836	0.7654	0.8359	101	39800	0.77 [0.68, 0.84]
Sp2	0.9864	0.01333	6.55E-05	0.9509	0.9905	0.9997	101	39800	0.99 [0.95, 0.99]
Sp3	0.9158	0.02529	1.24E-04	0.8602	0.9178	0.9585	101	39800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03212	1.09E-04	0.4978	0.5612	0.6233	101	79800	0.56 [0.50, 0.62]
Se1	0.978	0.00945	3.26E-05	0.9562	0.9793	0.9926	101	79800	0.98 [0.96, 0.99]
Se2	0.9859	0.01188	4.14E-05	0.9556	0.9889	0.9995	101	79800	0.99 [0.96, 0.99]
Se3	0.9526	0.01828	6.58E-05	0.9111	0.9547	0.9823	101	79800	0.95 [0.91, 0.98]
Sp1	0.7642	0.03894	1.33E-04	0.6843	0.7656	0.8362	101	79800	0.77 [0.68, 0.84]
Sp2	0.9864	0.01337	4.76E-05	0.951	0.9905	0.9996	101	79800	0.99 [0.95, 0.99]
Sp3	0.9157	0.02529	8.74E-05	0.8602	0.9179	0.9586	101	79800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03209	9.16E-05	0.4979	0.5612	0.6232	101	119800	0.56 [0.50, 0.62]
Se1	0.9779	0.0095	2.57E-05	0.956	0.9793	0.9926	101	119800	0.98 [0.96, 0.99]
Se2	0.9859	0.01189	3.48E-05	0.9555	0.9889	0.9995	101	119800	0.99 [0.96, 0.99]
Se3	0.9526	0.01831	5.28E-05	0.9111	0.9546	0.9822	101	119800	0.95 [0.91, 0.98]
Sp1	0.7641	0.03889	1.08E-04	0.6846	0.7655	0.8361	101	119800	0.77 [0.68, 0.84]
Sp2	0.9864	0.01338	3.71E-05	0.9509	0.9905	0.9997	101	119800	0.99 [0.95, 0.99]
Sp3	0.9157	0.02534	7.39E-05	0.8602	0.9178	0.9585	101	119800	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03207	8.02E-05	0.4978	0.5612	0.6232	101	159800	0.56 [0.50, 0.62]
Se1	0.9779	0.0095	2.26E-05	0.9559	0.9792	0.9926	101	159800	0.98 [0.96, 0.99]
Se2	0.9859	0.01189	2.93E-05	0.9556	0.9889	0.9995	101	159800	0.99 [0.96, 0.99]
Se3	0.9525	0.01831	4.73E-05	0.9111	0.9546	0.9822	101	159800	0.95 [0.91, 0.98]
Sp1	0.7641	0.03894	9.46E-05	0.6843	0.7655	0.8362	101	159800	0.77 [0.68, 0.84]
Sp2	0.9864	0.01335	3.29E-05	0.9508	0.9905	0.9996	101	159800	0.99 [0.95, 0.99]
Sp3	0.9157	0.02533	6.33E-05	0.8602	0.9179	0.9586	101	159800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03204	7.03E-05	0.4979	0.5612	0.6233	101	199800	0.56 [0.50, 0.62]
Se1	0.9779	0.0095	2.06E-05	0.9559	0.9792	0.9926	101	199800	0.98 [0.96, 0.99]
Se2	0.9859	0.01188	2.78E-05	0.9556	0.9889	0.9995	101	199800	0.99 [0.96, 0.99]
Se3	0.9526	0.0183	4.14E-05	0.9112	0.9546	0.9822	101	199800	0.95 [0.91, 0.98]
Sp1	0.7641	0.03893	8.63E-05	0.6842	0.7656	0.8362	101	199800	0.77 [0.68, 0.84]
Sp2	0.9864	0.01335	2.99E-05	0.9509	0.9905	0.9996	101	199800	0.99 [0.95, 0.99]
Sp3	0.9157	0.02533	5.47E-05	0.8602	0.9179	0.9587	101	199800	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03207	7.08E-05	0.4978	0.5612	0.6233	20000	200002	0.56 [0.50, 0.62]
Se1	0.9779	0.00951	2.08E-05	0.9558	0.9792	0.9926	20000	200002	0.98 [0.96, 0.99]
Se2	0.9859	0.01188	2.84E-05	0.9556	0.9889	0.9995	20000	200002	0.99 [0.96, 0.99]
Se3	0.9525	0.01829	4.10E-05	0.9113	0.9546	0.9822	20000	200002	0.95 [0.91, 0.98]
Sp1	0.7641	0.03896	8.59E-05	0.6841	0.7656	0.8362	20000	200002	0.77 [0.68, 0.84]
Sp2	0.9864	0.01335	2.93E-05	0.9508	0.9905	0.9996	20000	200002	0.99 [0.95, 0.99]
Sp3	0.9157	0.02535	5.53E-05	0.8599	0.9179	0.9587	20000	200002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03206	8.16E-05	0.4977	0.5612	0.6234	40000	160002	0.56 [0.50, 0.62]
Se1	0.9779	0.00952	2.33E-05	0.9558	0.9792	0.9926	40000	160002	0.98 [0.96, 0.99]
Se2	0.9859	0.01189	3.12E-05	0.9556	0.9889	0.9995	40000	160002	0.99 [0.96, 0.99]
Se3	0.9525	0.01831	4.55E-05	0.9113	0.9545	0.9822	40000	160002	0.95 [0.91, 0.98]
Sp1	0.764	0.03897	9.54E-05	0.6839	0.7655	0.8361	40000	160002	0.77 [0.68, 0.84]
Sp2	0.9864	0.01333	3.35E-05	0.9507	0.9905	0.9996	40000	160002	0.99 [0.95, 0.99]
Sp3	0.9157	0.02536	6.16E-05	0.8599	0.9179	0.9588	40000	160002	0.92 [0.86, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5611	0.03206	9.21E-05	0.4976	0.5612	0.6236	60000	120002	0.56 [0.05, 0.62]
Se1	0.9779	0.0095	2.82E-05	0.9558	0.9792	0.9926	60000	120002	0.98 [0.96, 0.99]
Se2	0.9859	0.01188	3.68E-05	0.9556	0.9889	0.9995	60000	120002	0.99 [0.96, 0.99]
Se3	0.9525	0.01829	5.40E-05	0.9115	0.9546	0.9823	60000	120002	0.95 [0.91, 0.98]
Sp1	0.764	0.03903	1.15E-04	0.6835	0.7656	0.8361	60000	120002	0.77 [0.68, 0.84]
Sp2	0.9864	0.01332	3.96E-05	0.9507	0.9905	0.9996	60000	120002	0.99 [0.95, 0.99]
Sp3	0.9157	0.02533	7.22E-05	0.8598	0.918	0.9588	60000	120002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5611	0.0321	1.13E-04	0.4976	0.5613	0.6237	80000	80002	0.56 [0.50, 0.62]
Se1	0.9779	0.0095	3.25E-05	0.9558	0.9792	0.9925	80000	80002	0.98 [0.96, 0.99]
Se2	0.9859	0.01189	4.39E-05	0.9557	0.9889	0.9995	80000	80002	0.99 [0.96, 0.99]
Se3	0.9526	0.01828	6.69E-05	0.9115	0.9546	0.9823	80000	80002	0.95 [0.91, 0.98]
Sp1	0.7641	0.03899	1.36E-04	0.6836	0.7657	0.8361	80000	80002	0.77 [0.68, 0.84]
Sp2	0.9864	0.01334	4.67E-05	0.9507	0.9905	0.9996	80000	80002	0.99 [0.95, 0.99]
Sp3	0.9157	0.02536	8.77E-05	0.8597	0.918	0.9588	80000	80002	0.92 [0.86, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.561	0.03228	1.59E-04	0.4972	0.5613	0.624	100000	40002	0.56 [0.50, 0.62]
Se1	0.9779	0.0095	4.51E-05	0.9559	0.9792	0.9926	100000	40002	0.98 [0.96, 0.99]
Se2	0.9859	0.01191	5.94E-05	0.9557	0.989	0.9995	100000	40002	0.99 [0.96, 0.99]
Se3	0.9525	0.01828	8.67E-05	0.9116	0.9546	0.9823	100000	40002	0.95 [0.91, 0.98]
Sp1	0.7639	0.0391	1.94E-04	0.6833	0.7654	0.8359	100000	40002	0.77 [0.68, 0.84]
Sp2	0.9864	0.01334	6.28E-05	0.9503	0.9905	0.9997	100000	40002	0.99 [0.95, 0.99]
Sp3	0.9157	0.02537	1.28E-04	0.8592	0.918	0.9587	100000	40002	0.92 [0.86, 0.96]

Pr1 = Prevalence of disease in the population.

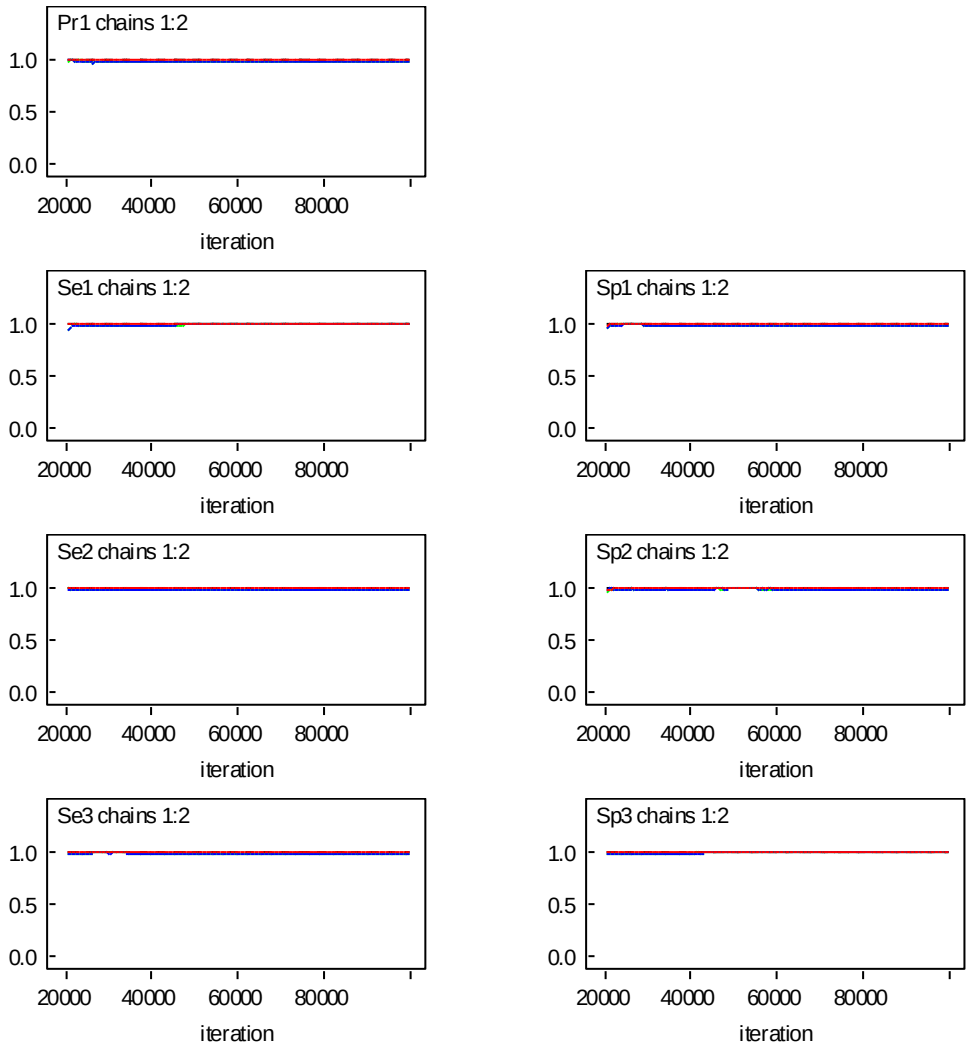
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.7.2: Plots of iterates for each variable to check for convergence for the data set for the mildly autolysed cases.



Pr1 = Prevalence of disease in the population.

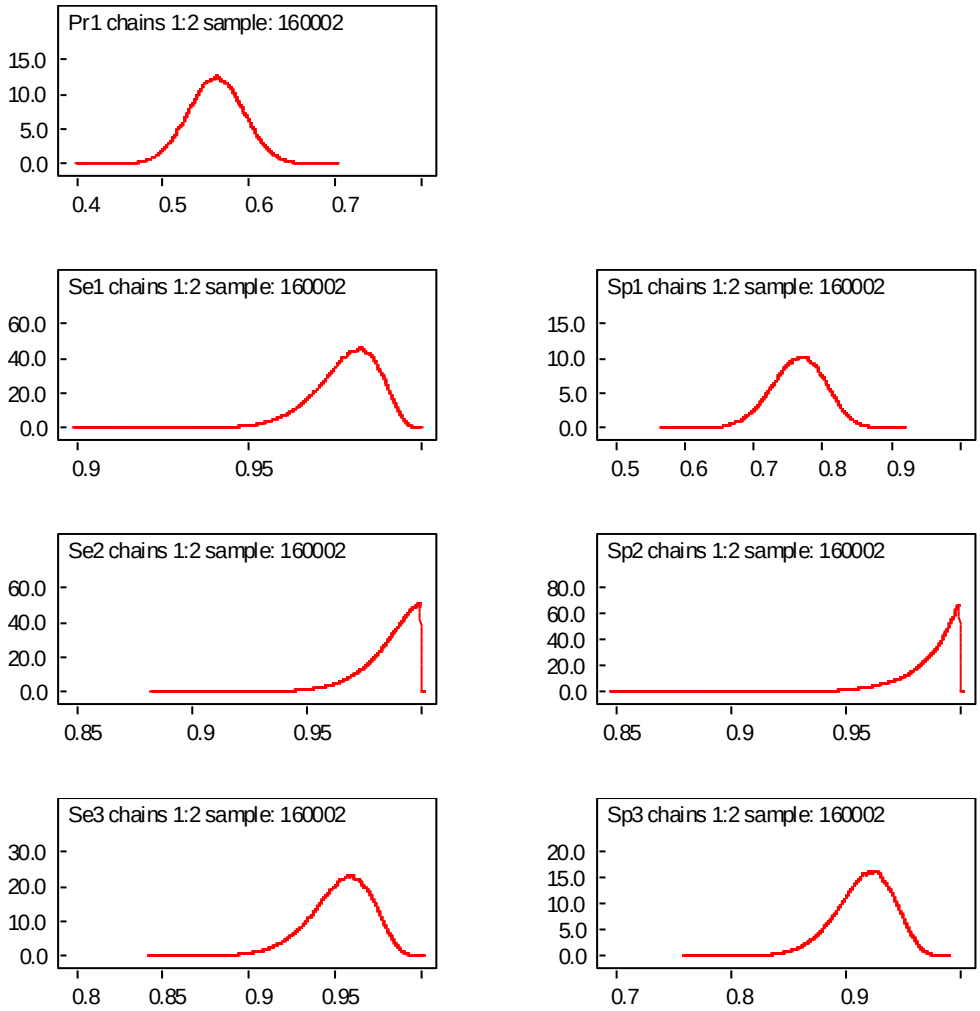
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.7.3: Kernel density estimate for the data set for the mildly autolysed cases.



Pr1 = Prevalence of disease in the population.

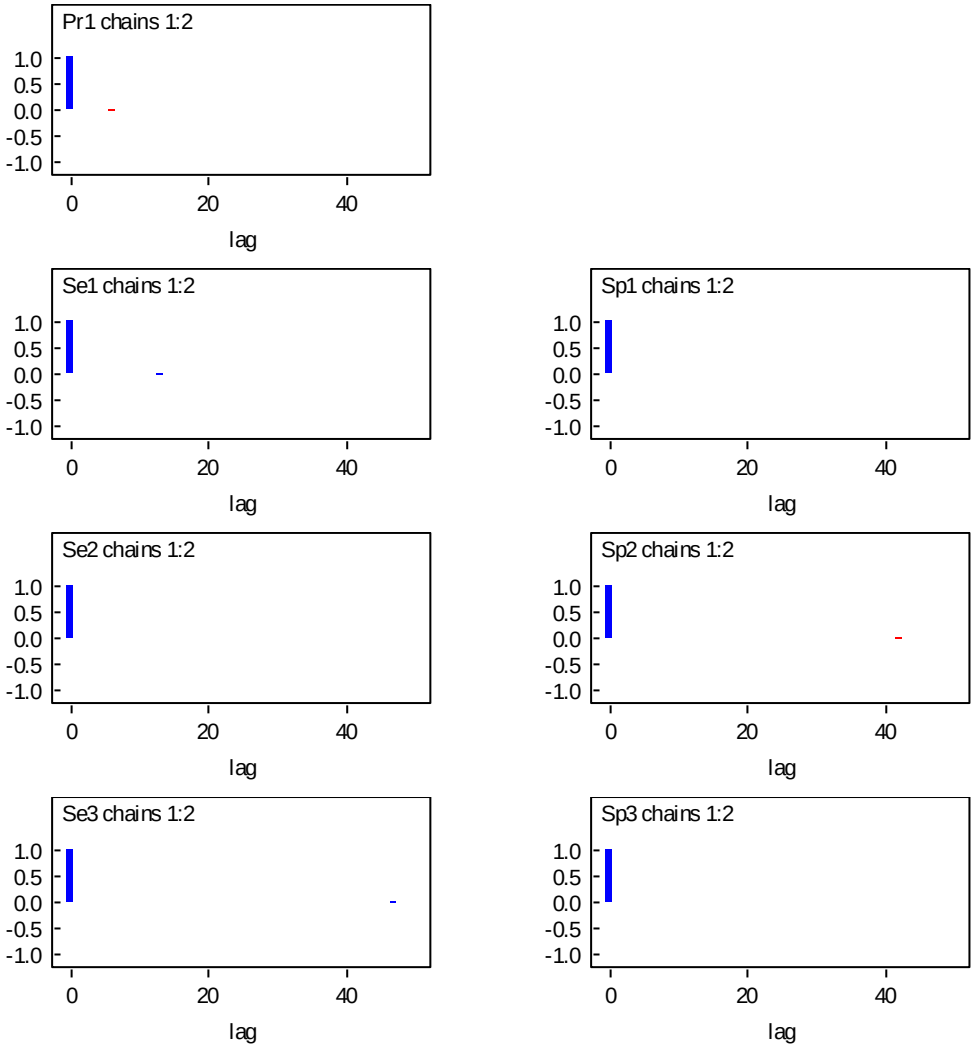
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.7.4: Autocorrelation plots for the data set for the mildly autolysed cases.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.8: Analysis of the data set for the 72 moderately autolysed cases.

Appendix 9.8.1: Posterior values obtained for each parameter for the data set for the moderately autolysed cases.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6283	0.05782	2.97E-04	0.5119	0.6297	0.7379	101	39800	0.63 [0.51, 0.74]
Se1	0.9722	0.01355	6.87E-05	0.9401	0.9742	0.9925	101	39800	0.97 [0.94, 0.99]
Se2	0.9764	0.02297	1.20E-04	0.9151	0.9834	0.9994	101	39800	0.98 [0.92, 0.99]
Se3	0.929	0.03739	1.89E-04	0.8413	0.9342	0.9866	101	39800	0.93 [0.84, 0.99]
Sp1	0.7456	0.06813	3.34E-04	0.6033	0.7496	0.8681	101	39800	0.75 [0.60, 0.87]
Sp2	0.9437	0.05113	2.59E-04	0.8112	0.9583	0.9984	101	39800	0.96 [0.81, 0.99]
Sp3	0.9012	0.0435	2.09E-04	0.8008	0.9073	0.9687	101	39800	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6282	0.05781	2.23E-04	0.5116	0.6296	0.7372	101	79800	0.63 [0.51, 0.74]
Se1	0.9722	0.01347	4.53E-05	0.9405	0.9742	0.9925	101	79800	0.97 [0.94, 0.99]
Se2	0.9764	0.02309	8.47E-05	0.9148	0.9834	0.9994	101	79800	0.98 [0.91, 0.99]
Se3	0.9289	0.03754	1.39E-04	0.8411	0.9342	0.9866	101	79800	0.93 [0.84, 0.99]
Sp1	0.7458	0.06815	2.45E-04	0.6028	0.7497	0.8677	101	79800	0.75 [0.60, 0.87]
Sp2	0.9435	0.05131	1.90E-04	0.8092	0.9581	0.9984	101	79800	0.96 [0.81, 0.99]
Sp3	0.9013	0.04354	1.49E-04	0.8012	0.9073	0.9686	101	79800	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6281	0.05773	1.63E-04	0.5114	0.6295	0.7371	101	119800	0.63 [0.51, 0.74]
Se1	0.9722	0.01348	3.87E-05	0.9405	0.9742	0.9925	101	119800	0.97 [0.94, 0.99]
Se2	0.9764	0.02307	6.67E-05	0.9149	0.9834	0.9994	101	119800	0.98 [0.91, 0.99]
Se3	0.9289	0.03747	1.14E-04	0.8416	0.9342	0.9866	101	119800	0.93 [0.84, 0.99]
Sp1	0.7459	0.06819	2.03E-04	0.6027	0.7498	0.8675	101	119800	0.75 [0.60, 0.87]
Sp2	0.9433	0.0514	1.51E-04	0.809	0.9579	0.9984	101	119800	0.96 [0.81, 0.99]
Sp3	0.9014	0.04352	1.29E-04	0.8014	0.9073	0.9688	101	119800	0.91 [0.80, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6281	0.05769	1.49E-04	0.5113	0.6294	0.7369	101	159800	0.63 [0.51, 0.74]
Se1	0.9722	0.01349	3.31E-05	0.9405	0.9742	0.9925	101	159800	0.97 [0.94, 0.99]
Se2	0.9764	0.02302	6.01E-05	0.9148	0.9834	0.9994	101	159800	0.98 [0.91, 0.99]
Se3	0.9289	0.03747	9.68E-05	0.8416	0.9342	0.9865	101	159800	0.93 [0.84, 0.99]
Sp1	0.7458	0.06822	1.69E-04	0.6025	0.7498	0.8671	101	159800	0.75 [0.60, 0.87]
Sp2	0.9432	0.05148	1.31E-04	0.8091	0.9579	0.9984	101	159800	0.96 [0.81, 0.99]
Sp3	0.9015	0.04348	1.06E-04	0.8017	0.9073	0.9688	101	159800	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.628	0.05761	1.28E-04	0.5115	0.6294	0.7367	101	199800	0.63 [0.51, 0.74]
Se1	0.9722	0.0135	2.98E-05	0.9405	0.9742	0.9925	101	199800	0.97 [0.94, 0.99]
Se2	0.9764	0.02301	5.47E-05	0.9149	0.9833	0.9994	101	199800	0.98 [0.91, 0.99]
Se3	0.929	0.03748	8.85E-05	0.8418	0.9343	0.9865	101	199800	0.93 [0.84, 0.99]
Sp1	0.7456	0.06815	1.51E-04	0.6026	0.7496	0.867	101	199800	0.75 [0.60, 0.87]
Sp2	0.9432	0.05147	1.19E-04	0.8091	0.9578	0.9984	101	199800	0.96 [0.81, 0.99]
Sp3	0.9014	0.04352	9.47E-05	0.8015	0.9072	0.9688	101	199800	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.628	0.05753	1.26E-04	0.5114	0.6293	0.7365	20000	200002	0.63 [0.51, 0.74]
Se1	0.9722	0.01349	2.98E-05	0.9405	0.9742	0.9925	20000	200002	0.97 [0.94, 0.99]
Se2	0.9764	0.02294	5.31E-05	0.9152	0.9833	0.9994	20000	200002	0.98 [0.92, 0.99]
Se3	0.929	0.0375	9.11E-05	0.8418	0.9343	0.9865	20000	200002	0.93 [0.84, 0.99]
Sp1	0.7458	0.06816	1.47E-04	0.6024	0.7496	0.8668	20000	200002	0.75 [0.60, 0.87]
Sp2	0.9431	0.05153	1.17E-04	0.809	0.9578	0.9984	20000	200002	0.96 [0.81, 0.99]
Sp3	0.9015	0.04353	9.52E-05	0.8018	0.9072	0.969	20000	200002	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6279	0.05746	1.41E-04	0.5114	0.6293	0.7364	40000	160002	0.63 [0.51, 0.74]
Se1	0.9721	0.01352	3.37E-05	0.9403	0.9741	0.9925	40000	160002	0.97 [0.94, 0.99]
Se2	0.9764	0.02287	6.04E-05	0.9154	0.9833	0.9994	40000	160002	0.98 [0.92, 0.99]
Se3	0.929	0.03745	1.01E-04	0.8421	0.9343	0.9865	40000	160002	0.93 [0.84, 0.99]
Sp1	0.7457	0.06816	1.62E-04	0.6026	0.7496	0.8667	40000	160002	0.75 [0.60, 0.87]
Sp2	0.9431	0.05154	1.29E-04	0.8093	0.9578	0.9984	40000	160002	0.96 [0.81, 0.99]
Sp3	0.9015	0.04352	1.10E-04	0.8019	0.9071	0.9692	40000	160002	0.91 [0.80, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6279	0.05742	1.64E-04	0.5116	0.6293	0.7364	60000	120002	0.63 [0.51, 0.74]
Se1	0.9721	0.01353	3.83E-05	0.9403	0.9742	0.9925	60000	120002	0.97 [0.94, 0.99]
Se2	0.9764	0.02282	6.85E-05	0.9156	0.9833	0.9994	60000	120002	0.98 [0.92, 0.99]
Se3	0.929	0.03748	1.10E-04	0.842	0.9343	0.9865	60000	120002	0.93 [0.84, 0.99]
Sp1	0.7456	0.06812	1.84E-04	0.6025	0.7495	0.8665	60000	120002	0.75 [0.60, 0.87]
Sp2	0.9431	0.05153	1.50E-04	0.8095	0.9579	0.9984	60000	120002	0.96 [0.81, 0.99]
Sp3	0.9015	0.04353	1.23E-04	0.8019	0.9071	0.9692	60000	120002	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.628	0.05736	2.09E-04	0.512	0.6294	0.7364	80000	80002	0.63 [0.51, 0.74]
Se1	0.9721	0.01353	4.72E-05	0.9402	0.9742	0.9925	80000	80002	0.97 [0.94, 0.99]
Se2	0.9764	0.02279	8.17E-05	0.916	0.9832	0.9994	80000	80002	0.98 [0.92, 0.99]
Se3	0.929	0.03749	1.37E-04	0.842	0.9344	0.9865	80000	80002	0.93 [0.84, 0.99]
Sp1	0.7457	0.06804	2.45E-04	0.6028	0.7493	0.8667	80000	80002	0.75 [0.60, 0.87]
Sp2	0.9432	0.05144	1.91E-04	0.8098	0.9579	0.9984	80000	80002	0.96 [0.81, 0.99]
Sp3	0.9013	0.04362	1.54E-04	0.8016	0.907	0.9693	80000	80002	0.91 [0.80, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.6282	0.05744	2.87E-04	0.5116	0.6295	0.7369	100000	40002	0.63 [0.51, 0.74]
Se1	0.9722	0.01351	6.69E-05	0.9402	0.9741	0.9926	100000	40002	0.97 [0.94, 0.99]
Se2	0.9765	0.02264	1.16E-04	0.9171	0.9832	0.9994	100000	40002	0.98 [0.92, 0.99]
Se3	0.9288	0.03745	2.01E-04	0.8417	0.9341	0.9864	100000	40002	0.93 [0.84, 0.99]
Sp1	0.7463	0.06817	3.50E-04	0.6027	0.7499	0.8671	100000	40002	0.75 [0.60, 0.87]
Sp2	0.9435	0.05143	2.86E-04	0.8104	0.9585	0.9984	100000	40002	0.96 [0.81, 0.99]
Sp3	0.9017	0.04354	2.15E-04	0.8023	0.9071	0.9695	100000	40002	0.91 [0.80, 0.97]

Pr1 = Prevalence of disease in the population.

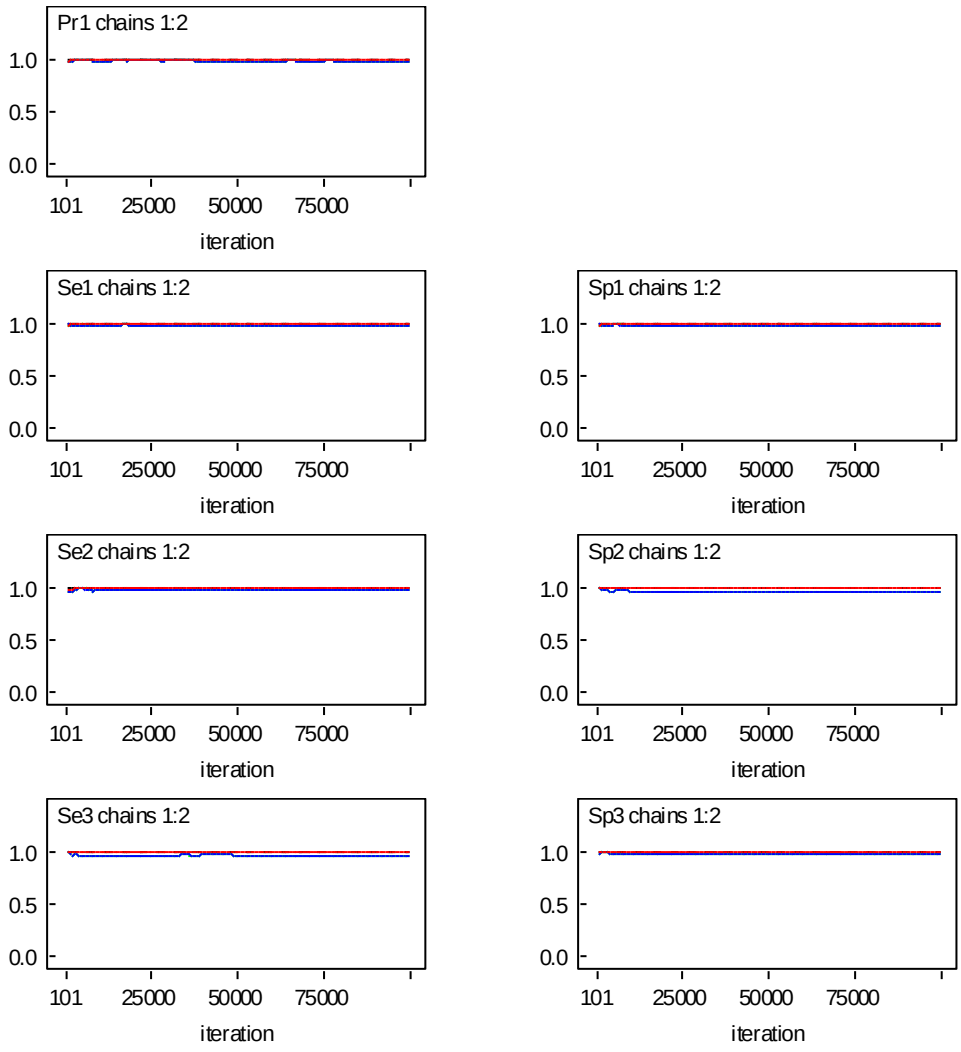
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.8.2: Plots of iterates for each variable to check for convergence for the data set for the moderately autolysed cases.



Pr1 = Prevalence of disease in the population.

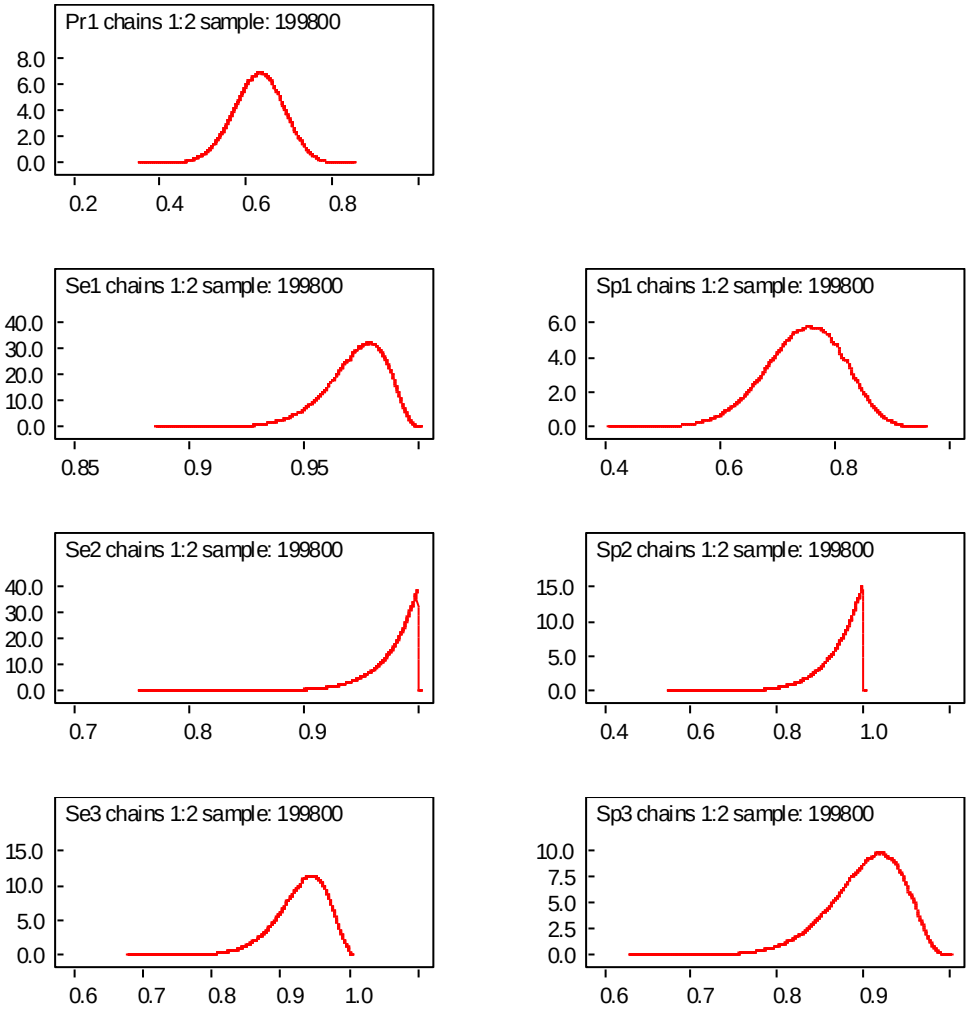
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.8.3: Kernel density estimate for the data set for the moderately autolysed cases.



Pr1 = Prevalence of disease in the population.

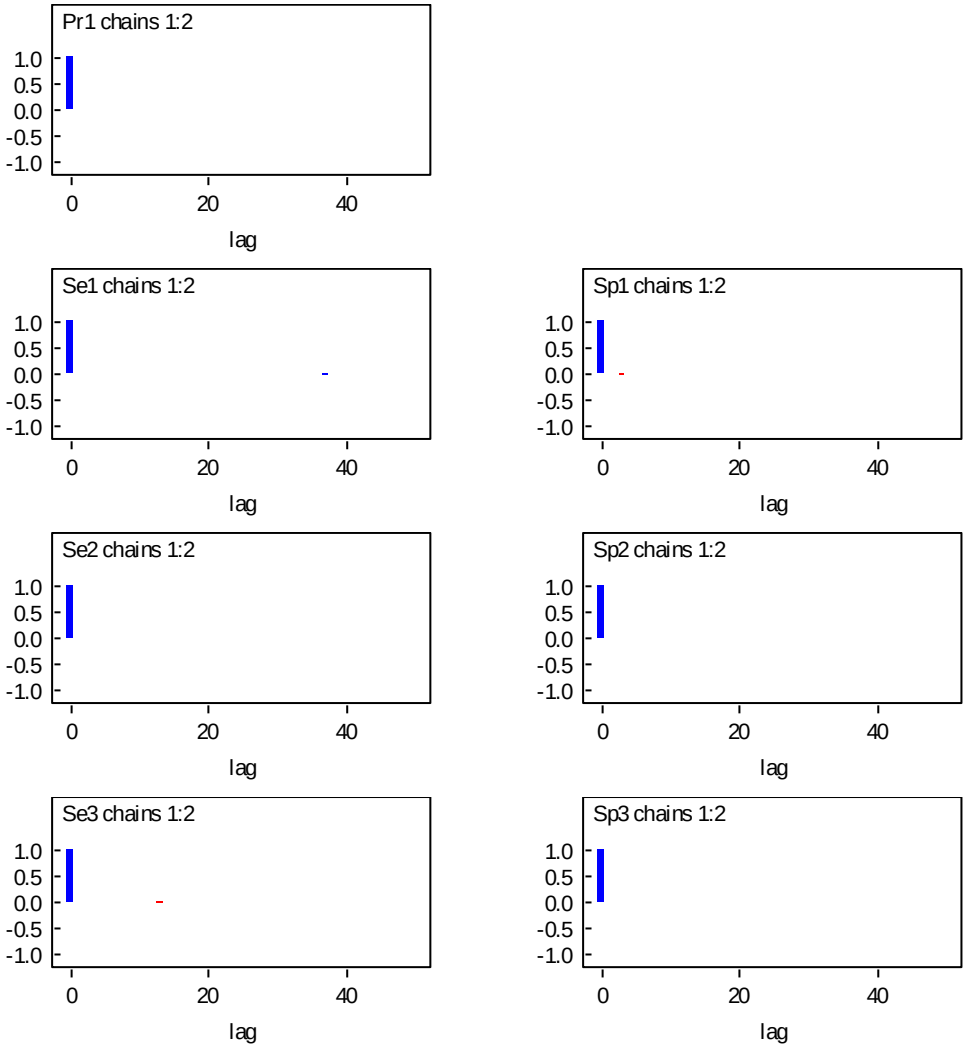
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.8.4: Autocorrelation plots for the data set for the moderately autolysed cases.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.9: Analysis of the data set for the 65 severely autolysed cases.

Appendix 9.9.1: Posterior values obtained for each parameter for the data set for the severely autolysed cases.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4119	0.07186	3.48E-04	0.2754	0.4107	0.5551	101	39800	0.41 [0.28, 0.56]
Se1	0.975	0.01385	6.93E-05	0.9418	0.9775	0.9949	101	39800	0.98 [0.94, 0.99]
Se2	0.8091	0.0953	4.56E-04	0.6085	0.8153	0.9747	101	39800	0.82 [0.61, 0.97]
Se3	0.911	0.05964	3.00E-04	0.7672	0.9217	0.9932	101	39800	0.92 [0.77, 0.99]
Sp1	0.5437	0.07218	3.66E-04	0.4039	0.5431	0.6843	101	39800	0.54 [0.40, 0.68]
Sp2	0.9511	0.04113	2.11E-04	0.8471	0.9614	0.9985	101	39800	0.96 [0.85, 0.99]
Sp3	0.8685	0.05026	2.53E-04	0.7582	0.8734	0.9519	101	39800	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4124	0.07209	2.34E-04	0.2758	0.4113	0.5564	101	79800	0.41 [0.28, 0.56]
Se1	0.9751	0.01384	4.91E-05	0.9418	0.9774	0.9949	101	79800	0.98 [0.94, 0.99]
Se2	0.8089	0.09543	3.22E-04	0.6082	0.8148	0.975	101	79800	0.81 [0.61, 0.98]
Se3	0.911	0.05982	2.09E-04	0.7677	0.9218	0.9934	101	79800	0.92 [0.77, 0.99]
Sp1	0.5434	0.07209	2.44E-04	0.404	0.5428	0.6842	101	79800	0.54 [0.40, 0.68]
Sp2	0.9513	0.04117	1.52E-04	0.8471	0.9616	0.9984	101	79800	0.96 [0.85, 0.99]
Sp3	0.8684	0.05043	1.74E-04	0.7576	0.8733	0.9519	101	79800	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4124	0.07216	2.07E-04	0.2755	0.4111	0.5569	101	119800	0.41 [0.28, 0.56]
Se1	0.9751	0.01383	4.02E-05	0.9419	0.9775	0.9949	101	119800	0.98 [0.94, 0.99]
Se2	0.8087	0.0958	2.80E-04	0.6066	0.8145	0.9752	101	119800	0.81 [0.61, 0.98]
Se3	0.9108	0.0598	1.70E-04	0.7675	0.9214	0.9933	101	119800	0.92 [0.77, 0.99]
Sp1	0.5435	0.07224	2.09E-04	0.4037	0.5428	0.6849	101	119800	0.54 [0.40, 0.68]
Sp2	0.9512	0.04121	1.20E-04	0.8472	0.9617	0.9984	101	119800	0.96 [0.85, 0.99]
Sp3	0.8686	0.05036	1.48E-04	0.758	0.8734	0.9523	101	119800	0.87 [0.76, 0.95]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4123	0.0722	1.80E-04	0.2754	0.4112	0.5567	101	159800	0.41 [0.28, 0.56]
Se1	0.9751	0.01384	3.56E-05	0.9418	0.9775	0.9949	101	159800	0.98 [0.94, 0.99]
Se2	0.8087	0.09572	2.46E-04	0.6063	0.8146	0.9753	101	159800	0.81 [0.61, 0.98]
Se3	0.9106	0.05987	1.46E-04	0.7672	0.9213	0.9933	101	159800	0.92 [0.77, 0.99]
Sp1	0.5435	0.07218	1.88E-04	0.4036	0.543	0.6846	101	159800	0.54 [0.40, 0.68]
Sp2	0.9512	0.04117	1.07E-04	0.8472	0.9616	0.9984	101	159800	0.96 [0.85, 0.99]
Sp3	0.8686	0.05033	1.26E-04	0.7582	0.8734	0.9523	101	159800	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4123	0.07218	1.60E-04	0.2755	0.4111	0.5566	101	199800	0.41 [0.28, 0.56]
Se1	0.9751	0.01385	3.16E-05	0.9419	0.9775	0.9949	101	199800	0.98 [0.94, 0.99]
Se2	0.8087	0.0957	2.10E-04	0.6061	0.8145	0.9753	101	199800	0.81 [0.61, 0.98]
Se3	0.9106	0.05995	1.37E-04	0.7672	0.9212	0.9933	101	199800	0.92 [0.77, 0.99]
Sp1	0.5434	0.07221	1.62E-04	0.4034	0.5431	0.685	101	199800	0.54 [0.40, 0.69]
Sp2	0.9512	0.04121	9.66E-05	0.8472	0.9616	0.9984	101	199800	0.96 [0.85, 0.99]
Sp3	0.8686	0.05042	1.16E-04	0.758	0.8734	0.9523	101	199800	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4124	0.0722	1.60E-04	0.2752	0.4112	0.5567	20000	200002	0.41 [0.28, 0.56]
Se1	0.9751	0.01387	3.05E-05	0.9418	0.9775	0.9949	20000	200002	0.98 [0.94, 0.99]
Se2	0.8086	0.09587	2.13E-04	0.6053	0.8144	0.9754	20000	200002	0.81 [0.61, 0.98]
Se3	0.9105	0.05992	1.34E-04	0.7674	0.9211	0.9932	20000	200002	0.92 [0.77, 0.99]
Sp1	0.5434	0.07223	1.63E-04	0.4032	0.5431	0.6851	20000	200002	0.54 [0.40, 0.69]
Sp2	0.9512	0.04121	9.90E-05	0.8471	0.9616	0.9984	20000	200002	0.96 [0.85, 0.99]
Sp3	0.8687	0.05039	1.18E-04	0.758	0.8735	0.9525	20000	200002	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4122	0.07217	1.83E-04	0.2749	0.411	0.5565	40000	160002	0.41 [0.27, 0.56]
Se1	0.9751	0.01388	3.45E-05	0.9418	0.9775	0.9949	40000	160002	0.98 [0.94, 0.99]
Se2	0.8086	0.09594	2.35E-04	0.6048	0.8144	0.9754	40000	160002	0.81 [0.60, 0.98]
Se3	0.9104	0.05991	1.46E-04	0.7672	0.9209	0.9931	40000	160002	0.92 [0.77, 0.99]
Sp1	0.5435	0.07229	1.84E-04	0.4029	0.5433	0.6853	40000	160002	0.54 [0.40, 0.69]
Sp2	0.9511	0.04121	1.05E-04	0.8471	0.9616	0.9984	40000	160002	0.96 [0.85, 0.99]
Sp3	0.8688	0.05034	1.34E-04	0.7582	0.8735	0.9526	40000	160002	0.87 [0.76, 0.95]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4122	0.07213	2.05E-04	0.275	0.4111	0.5559	60000	120002	0.41 [0.28, 0.56]
Se1	0.9751	0.01391	4.00E-05	0.9417	0.9775	0.9948	60000	120002	0.98 [0.94, 0.99]
Se2	0.8087	0.09575	2.77E-04	0.6052	0.8146	0.9754	60000	120002	0.81 [0.61, 0.98]
Se3	0.9105	0.05995	1.72E-04	0.7672	0.9209	0.9931	60000	120002	0.92 [0.77, 0.99]
Sp1	0.5435	0.07221	2.08E-04	0.403	0.5435	0.6851	60000	120002	0.54 [0.40, 0.69]
Sp2	0.9511	0.04118	1.24E-04	0.847	0.9615	0.9984	60000	120002	0.96 [0.85, 0.99]
Sp3	0.8688	0.05038	1.54E-04	0.7581	0.8735	0.9525	60000	120002	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4122	0.07203	2.52E-04	0.275	0.411	0.5558	80000	80002	0.41 [0.28, 0.56]
Se1	0.9751	0.01392	4.75E-05	0.9418	0.9775	0.9949	80000	80002	0.98 [0.94, 0.99]
Se2	0.8086	0.09588	3.26E-04	0.6057	0.8144	0.9755	80000	80002	0.81 [0.61, 0.98]
Se3	0.9105	0.05989	2.03E-04	0.7679	0.921	0.993	80000	80002	0.92 [0.77, 0.99]
Sp1	0.5434	0.0723	2.49E-04	0.4027	0.5433	0.6858	80000	80002	0.54 [0.40, 0.69]
Sp2	0.9511	0.04125	1.57E-04	0.8471	0.9616	0.9984	80000	80002	0.96 [0.85, 0.99]
Sp3	0.8688	0.05045	1.87E-04	0.7577	0.8735	0.9526	80000	80002	0.87 [0.76, 0.95]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4123	0.07199	3.68E-04	0.274	0.4113	0.5557	100000	40002	0.41 [0.27, 0.56]
Se1	0.9751	0.01396	7.06E-05	0.9416	0.9775	0.9948	100000	40002	0.98 [0.94, 0.99]
Se2	0.8086	0.09612	4.73E-04	0.6057	0.8147	0.9751	100000	40002	0.81 [0.61, 0.98]
Se3	0.9106	0.05951	2.92E-04	0.7689	0.9209	0.9928	100000	40002	0.92 [0.77, 0.99]
Sp1	0.5437	0.07228	3.63E-04	0.4031	0.5434	0.6852	100000	40002	0.54 [0.40, 0.69]
Sp2	0.9512	0.04116	2.11E-04	0.8468	0.9618	0.9984	100000	40002	0.96 [0.85, 0.99]
Sp3	0.8691	0.05012	2.67E-04	0.7584	0.8737	0.9526	100000	40002	0.87 [0.76, 0.95]

Pr1 = Prevalence of disease in the population.

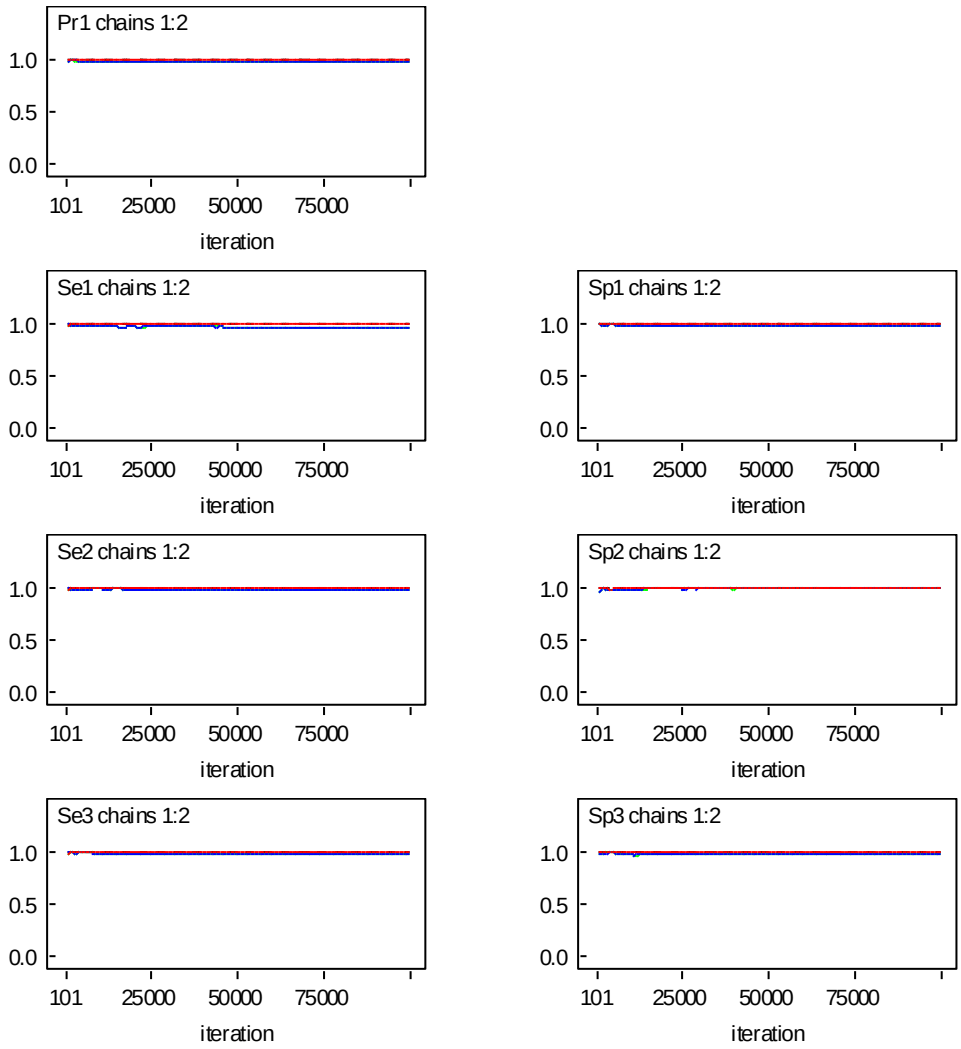
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.9.2: Plots of iterates for each variable to check for convergence for the data set for the severely autolysed cases.



Pr1 = Prevalence of disease in the population.

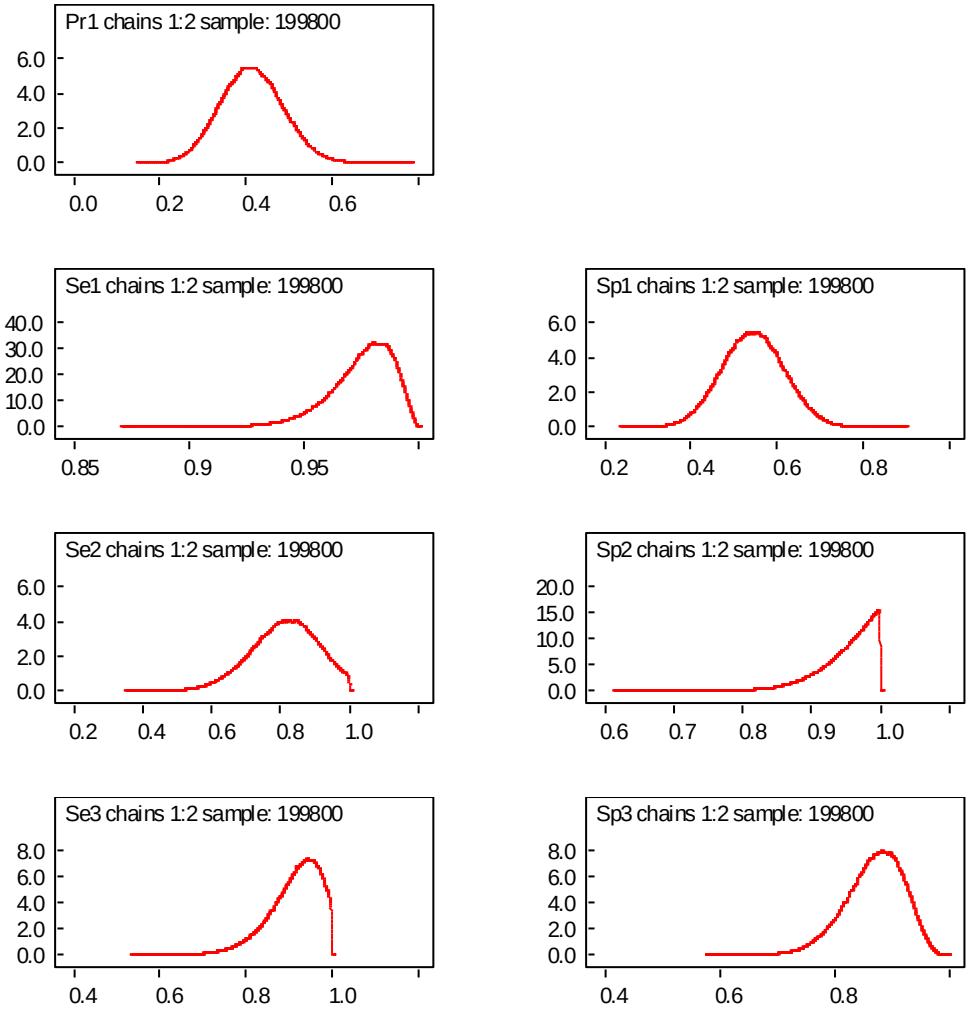
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.9.3: Kernel density estimate for the data set for the severely autolysed cases.



Pr1 = Prevalence of disease in the population.

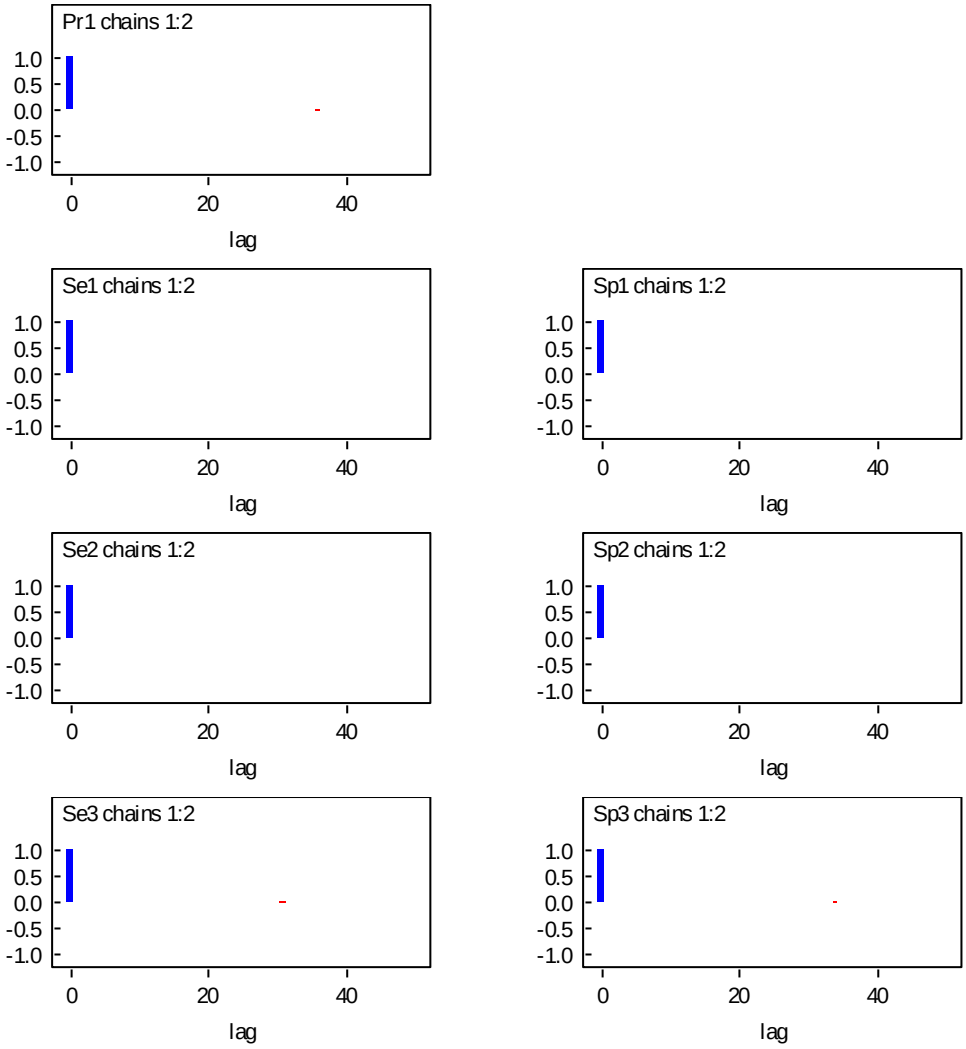
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.9.4: Autocorrelation plots for the data set for the severely autolysed cases.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.10: Analysis of the data set for the 100 cases where tissue pigments were present.

Appendix 9.10.1: Posterior values obtained for each parameter for the data set for the cases where tissue pigments were present.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5647	0.0511	2.61E-04	0.4642	0.5653	0.663	101	39800	0.57 [0.46, 0.66]
Se1	0.9516	0.02764	1.55E-04	0.8842	0.9566	0.9906	101	39800	0.96 [0.88, 0.99]
Se2	0.9273	0.03761	1.80E-04	0.8406	0.9323	0.9852	101	39800	0.93 [0.84, 0.99]
Se3	0.9149	0.02878	1.47E-04	0.8506	0.9177	0.9626	101	39800	0.92 [0.85, 0.96]
Sp1	0.7261	0.06372	3.38E-04	0.5931	0.7289	0.8426	101	39800	0.73 [0.59, 0.84]
Sp2	0.9763	0.02278	1.17E-04	0.9155	0.9831	0.9994	101	39800	0.98 [0.92, 0.99]
Sp3	0.8944	0.0462	2.35E-04	0.7915	0.8999	0.9693	101	39800	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.565	0.05123	1.83E-04	0.4638	0.5653	0.6639	101	79800	0.57 [0.46, 0.66]
Se1	0.9514	0.02758	9.67E-05	0.8849	0.9564	0.9904	101	79800	0.96 [0.88, 0.99]
Se2	0.9273	0.03758	1.35E-04	0.8407	0.9324	0.9849	101	79800	0.93 [0.84, 0.98]
Se3	0.9149	0.02889	1.04E-04	0.8503	0.9178	0.9628	101	79800	0.92 [0.85, 0.96]
Sp1	0.7262	0.06355	2.24E-04	0.5943	0.729	0.8419	101	79800	0.73 [0.59, 0.84]
Sp2	0.9764	0.02277	7.93E-05	0.9157	0.9832	0.9994	101	79800	0.98 [0.92, 0.99]
Sp3	0.8943	0.04632	1.64E-04	0.7904	0.8995	0.9695	101	79800	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5649	0.05131	1.53E-04	0.4631	0.5654	0.6641	101	119800	0.57 [0.46, 0.66]
Se1	0.9514	0.02763	8.16E-05	0.885	0.9564	0.9904	101	119800	0.96 [0.89, 0.99]
Se2	0.9273	0.0376	1.09E-04	0.8405	0.9323	0.9849	101	119800	0.93 [0.84, 0.98]
Se3	0.9149	0.02888	8.00E-05	0.8505	0.9177	0.9628	101	119800	0.92 [0.85, 0.96]
Sp1	0.7262	0.06328	1.83E-04	0.5953	0.7288	0.8418	101	119800	0.73 [0.60, 0.84]
Sp2	0.9764	0.02275	6.38E-05	0.9157	0.9833	0.9994	101	119800	0.98 [0.92, 0.99]
Sp3	0.8943	0.04629	1.36E-04	0.7903	0.8994	0.9693	101	119800	0.90 [0.79, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5648	0.05128	1.24E-04	0.4632	0.5653	0.6639	101	159800	0.57 [0.46, 0.66]
Se1	0.9514	0.02765	6.87E-05	0.8847	0.9563	0.9904	101	159800	0.96 [0.88, 0.99]
Se2	0.9274	0.03749	1.02E-04	0.8408	0.9324	0.9849	101	159800	0.93 [0.84, 0.98]
Se3	0.915	0.02886	6.88E-05	0.8507	0.9178	0.9629	101	159800	0.92 [0.85, 0.96]
Sp1	0.7262	0.06323	1.55E-04	0.5953	0.7286	0.8417	101	159800	0.73 [0.60, 0.84]
Sp2	0.9764	0.02281	5.75E-05	0.9157	0.9832	0.9994	101	159800	0.98 [0.92, 0.99]
Sp3	0.8943	0.04622	1.12E-04	0.7903	0.8994	0.9691	101	159800	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5648	0.0513	1.11E-04	0.4631	0.5653	0.6639	101	199800	0.57 [0.46, 0.66]
Se1	0.9514	0.02766	6.38E-05	0.8847	0.9563	0.9904	101	199800	0.96 [0.88, 0.99]
Se2	0.9274	0.03752	8.64E-05	0.8408	0.9325	0.9849	101	199800	0.93 [0.84, 0.98]
Se3	0.915	0.02887	6.31E-05	0.8507	0.9178	0.9629	101	199800	0.92 [0.85, 0.96]
Sp1	0.7261	0.06322	1.40E-04	0.5952	0.7287	0.8415	101	199800	0.73 [0.60, 0.84]
Sp2	0.9764	0.0228	5.08E-05	0.9157	0.9832	0.9994	101	199800	0.98 [0.92, 0.99]
Sp3	0.8943	0.04617	1.01E-04	0.7903	0.8994	0.9691	101	199800	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5648	0.05133	1.10E-04	0.4631	0.5653	0.6642	20000	200002	0.57 [0.46, 0.66]
Se1	0.9513	0.02768	6.22E-05	0.8848	0.9562	0.9903	20000	200002	0.96 [0.88, 0.99]
Se2	0.9274	0.03754	8.64E-05	0.8407	0.9325	0.9848	20000	200002	0.93 [0.84, 0.98]
Se3	0.915	0.02887	6.47E-05	0.8507	0.9179	0.9629	20000	200002	0.92 [0.85, 0.96]
Sp1	0.7261	0.06305	1.36E-04	0.5959	0.7285	0.8415	20000	200002	0.73 [0.60, 0.84]
Sp2	0.9764	0.02279	4.98E-05	0.9157	0.9833	0.9994	20000	200002	0.98 [0.92, 0.99]
Sp3	0.8943	0.04613	1.05E-04	0.7902	0.8993	0.969	20000	200002	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5647	0.05132	1.27E-04	0.463	0.5653	0.664	40000	160002	0.57 [0.46, 0.66]
Se1	0.9513	0.02772	7.18E-05	0.8846	0.9562	0.9903	40000	160002	0.96 [0.88, 0.99]
Se2	0.9274	0.03754	9.88E-05	0.8407	0.9325	0.9849	40000	160002	0.93 [0.84, 0.98]
Se3	0.915	0.02883	7.20E-05	0.8509	0.9179	0.9629	40000	160002	0.92 [0.85, 0.96]
Sp1	0.726	0.06296	1.47E-04	0.5961	0.7284	0.8415	40000	160002	0.73 [0.60, 0.84]
Sp2	0.9764	0.0228	5.75E-05	0.9157	0.9833	0.9994	40000	160002	0.98 [0.92, 0.99]
Sp3	0.8944	0.04605	1.13E-04	0.7905	0.8994	0.9689	40000	160002	0.90 [0.79, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5647	0.05128	1.45E-04	0.4634	0.5652	0.6639	60000	120002	0.57 [0.46, 0.66]
Se1	0.9513	0.02771	7.82E-05	0.8844	0.9562	0.9902	60000	120002	0.96 [0.88, 0.99]
Se2	0.9275	0.0375	1.11E-04	0.8409	0.9326	0.9849	60000	120002	0.93 [0.84, 0.98]
Se3	0.915	0.02882	8.31E-05	0.851	0.918	0.9629	60000	120002	0.92 [0.85, 0.96]
Sp1	0.726	0.06303	1.81E-04	0.5959	0.7283	0.8415	60000	120002	0.73 [0.60, 0.84]
Sp2	0.9763	0.02283	6.77E-05	0.9157	0.9832	0.9994	60000	120002	0.98 [0.92, 0.99]
Sp3	0.8944	0.046	1.30E-04	0.7906	0.8994	0.9688	60000	120002	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5648	0.05132	1.82E-04	0.4635	0.5653	0.6642	80000	80002	0.57 [0.46, 0.66]
Se1	0.9512	0.02771	1.00E-04	0.8846	0.9562	0.9903	80000	80002	0.96 [0.88, 0.99]
Se2	0.9274	0.03767	1.30E-04	0.8404	0.9326	0.9849	80000	80002	0.93 [0.84, 0.98]
Se3	0.915	0.02884	1.06E-04	0.8507	0.918	0.9628	80000	80002	0.92 [0.85, 0.96]
Sp1	0.7259	0.06302	2.17E-04	0.5961	0.7284	0.8416	80000	80002	0.73 [0.60, 0.84]
Sp2	0.9764	0.02277	8.34E-05	0.9156	0.9832	0.9994	80000	80002	0.98 [0.92, 0.99]
Sp3	0.8944	0.04599	1.60E-04	0.7906	0.8993	0.9691	80000	80002	0.90 [0.79, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5649	0.05126	2.49E-04	0.4642	0.565	0.6646	100000	40002	0.57 [0.46, 0.66]
Se1	0.9513	0.02773	1.45E-04	0.8846	0.9562	0.9903	100000	40002	0.96 [0.88, 0.99]
Se2	0.9274	0.03768	1.89E-04	0.8402	0.9326	0.9847	100000	40002	0.93 [0.84, 0.98]
Se3	0.9151	0.02877	1.49E-04	0.8507	0.9181	0.9627	100000	40002	0.92 [0.85, 0.96]
Sp1	0.726	0.06287	3.26E-04	0.5973	0.7282	0.8423	100000	40002	0.73 [0.60, 0.84]
Sp2	0.9764	0.02277	1.15E-04	0.9155	0.9832	0.9994	100000	40002	0.98 [0.92, 0.99]
Sp3	0.8946	0.04602	2.42E-04	0.791	0.8996	0.9691	100000	40002	0.90 [0.79, 0.97]

Pr1 = Prevalence of disease in the population

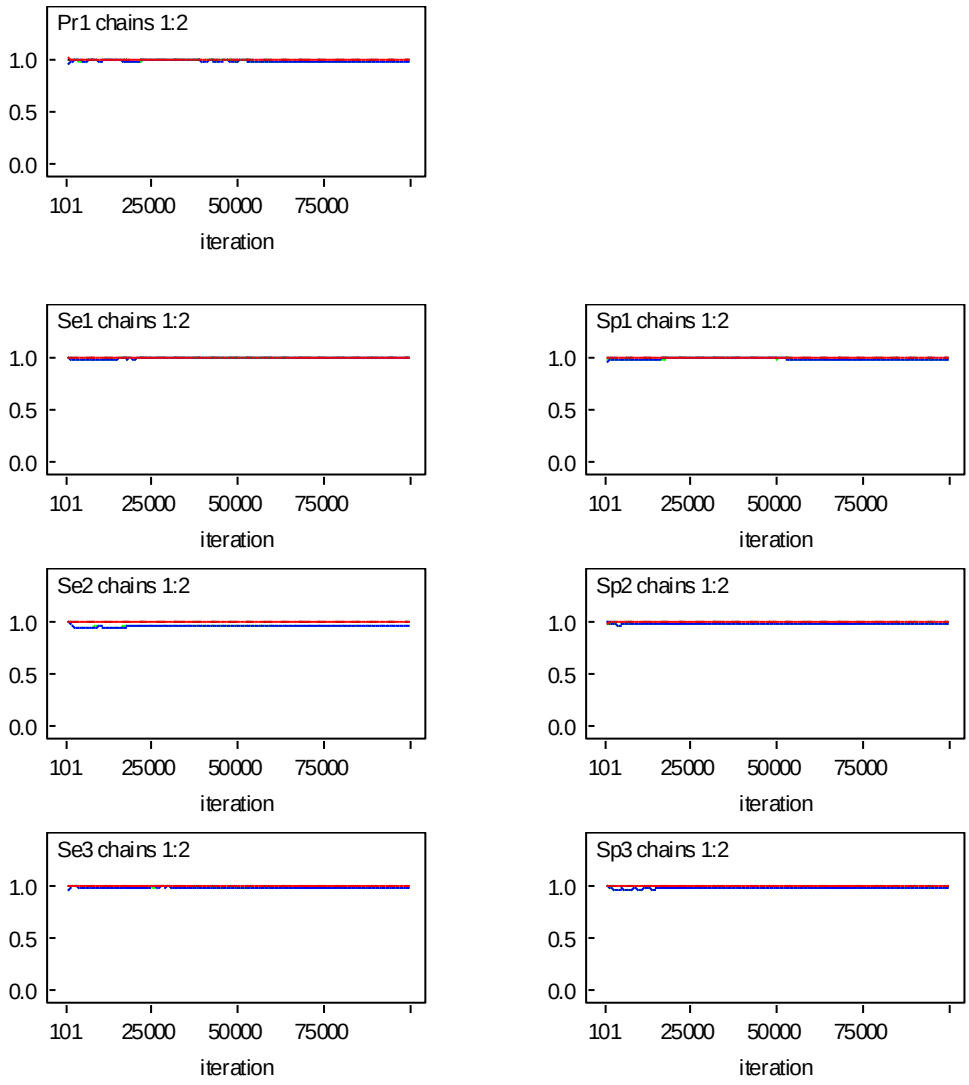
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.10.2: Plots of iterates for each variable to check for convergence for the data set for the cases where tissue pigments were present.



Pr1 = Prevalence of disease in the population.

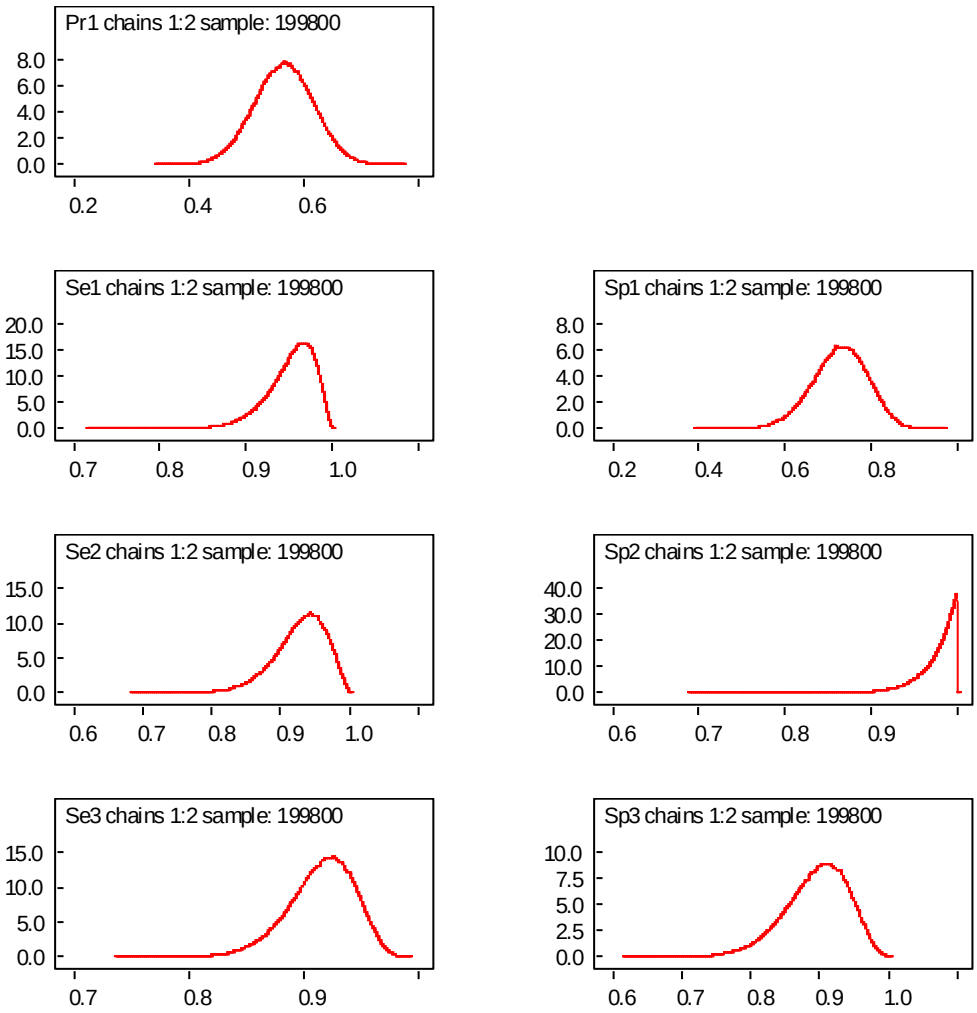
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.10.3: Kernel density estimate for the data set for the cases where tissue pigments were present.



Pr1 = Prevalence of disease in the population.

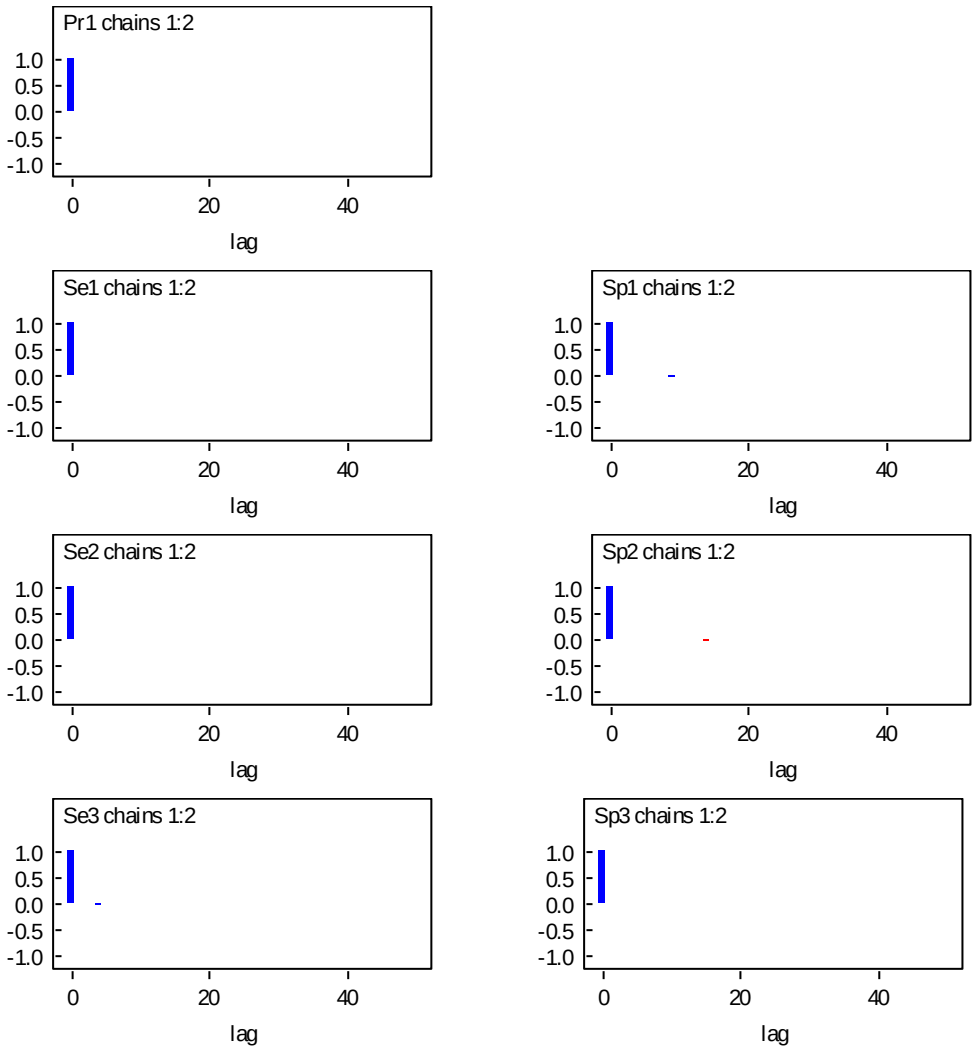
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.10.4: Autocorrelation plots for the data set for the cases where tissue pigments were present.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.11: Analysis of the data set for the 280 cases where tissue pigments were absent.

Appendix 9.11.1: Posterior values obtained for each parameter for the data set for the cases where tissue pigments were absent.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5469	0.03054	1.50E-04	0.4868	0.5468	0.6062	101	39800	0.55 [0.49, 0.61]
Se1	0.9716	0.01038	5.43E-05	0.9475	0.9727	0.9882	101	39800	0.97 [0.95, 0.99]
Se2	0.9753	0.01642	8.38E-05	0.9371	0.9779	0.9985	101	39800	0.98 [0.94, 0.99]
Se3	0.9452	0.01881	9.60E-05	0.9037	0.947	0.9768	101	39800	0.95 [0.90, 0.98]
Sp1	0.697	0.03955	1.95E-04	0.6178	0.6977	0.7719	101	39800	0.70 [0.62, 0.77]
Sp2	0.9873	0.01253	6.03E-05	0.9533	0.991	0.9997	101	39800	0.99 [0.95, 0.99]
Sp3	0.9254	0.02369	1.31E-04	0.8739	0.9275	0.9656	101	39800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5469	0.03051	1.07E-04	0.4867	0.5469	0.6063	101	79800	0.55 [0.49, 0.61]
Se1	0.9716	0.01041	3.74E-05	0.9477	0.9728	0.9882	101	79800	0.97 [0.95, 0.99]
Se2	0.9753	0.01643	5.88E-05	0.9368	0.9779	0.9985	101	79800	0.98 [0.94, 0.99]
Se3	0.9452	0.01879	6.83E-05	0.9038	0.947	0.9769	101	79800	0.95 [0.90, 0.98]
Sp1	0.697	0.03962	1.47E-04	0.6167	0.6979	0.7718	101	79800	0.70 [0.62, 0.77]
Sp2	0.9873	0.01253	4.31E-05	0.9536	0.9911	0.9997	101	79800	0.99 [0.95, 0.99]
Sp3	0.9255	0.02366	8.56E-05	0.8738	0.9276	0.9658	101	79800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5469	0.03049	9.08E-05	0.4866	0.547	0.606	101	119800	0.55 [0.49, 0.61]
Se1	0.9716	0.0104	2.97E-05	0.9478	0.9728	0.9882	101	119800	0.97 [0.95, 0.99]
Se2	0.9754	0.01638	4.84E-05	0.9369	0.9779	0.9985	101	119800	0.98 [0.94, 0.99]
Se3	0.9453	0.01879	5.55E-05	0.9038	0.947	0.9769	101	119800	0.95 [0.90, 0.98]
Sp1	0.6971	0.0396	1.12E-04	0.6169	0.6979	0.7721	101	119800	0.70 [0.62, 0.77]
Sp2	0.9873	0.01248	3.60E-05	0.954	0.9911	0.9997	101	119800	0.99 [0.95, 0.99]
Sp3	0.9255	0.02364	7.03E-05	0.8739	0.9276	0.9657	101	119800	0.93 [0.87, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5469	0.03048	7.50E-05	0.4868	0.547	0.6062	101	159800	0.55 [0.49, 0.61]
Se1	0.9716	0.0104	2.56E-05	0.9478	0.9728	0.9882	101	159800	0.97 [0.95, 0.99]
Se2	0.9754	0.01642	4.14E-05	0.9368	0.978	0.9985	101	159800	0.98 [0.94, 0.99]
Se3	0.9452	0.01877	4.70E-05	0.9038	0.947	0.9769	101	159800	0.95 [0.90, 0.98]
Sp1	0.697	0.03956	9.74E-05	0.6169	0.6979	0.772	101	159800	0.70 [0.62, 0.77]
Sp2	0.9873	0.01248	3.07E-05	0.9539	0.9911	0.9997	101	159800	0.99 [0.95, 0.99]
Sp3	0.9255	0.02367	6.09E-05	0.8739	0.9276	0.9658	101	159800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5469	0.0305	6.93E-05	0.4868	0.547	0.6062	101	199800	0.55 [0.49, 0.61]
Se1	0.9716	0.0104	2.26E-05	0.9479	0.9728	0.9882	101	199800	0.97 [0.95, 0.99]
Se2	0.9753	0.01643	3.59E-05	0.9367	0.9779	0.9985	101	199800	0.98 [0.94, 0.99]
Se3	0.9452	0.01879	4.24E-05	0.9038	0.947	0.9769	101	199800	0.95 [0.90, 0.98]
Sp1	0.6971	0.03959	8.71E-05	0.6168	0.6979	0.7721	101	199800	0.07 [0.62, 0.77]
Sp2	0.9873	0.01249	2.80E-05	0.9538	0.9911	0.9997	101	199800	0.99 [0.95, 0.99]
Sp3	0.9255	0.02366	5.29E-05	0.8738	0.9276	0.9658	101	199800	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5468	0.03049	7.09E-05	0.4867	0.5469	0.606	20000	200002	0.55 [0.49, 0.61]
Se1	0.9715	0.0104	2.24E-05	0.948	0.9727	0.9882	20000	200002	0.97 [0.95, 0.99]
Se2	0.9754	0.01641	3.65E-05	0.9367	0.978	0.9985	20000	200002	0.98 [0.94, 0.99]
Se3	0.9452	0.01881	4.13E-05	0.9036	0.947	0.9769	20000	200002	0.95 [0.90, 0.98]
Sp1	0.697	0.03957	8.60E-05	0.6169	0.6979	0.7719	20000	200002	0.70 [0.62, 0.77]
Sp2	0.9873	0.0125	2.79E-05	0.9538	0.9911	0.9997	20000	200002	0.99 [0.95, 0.99]
Sp3	0.9255	0.02368	5.18E-05	0.8738	0.9276	0.9658	20000	200002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5467	0.03049	7.66E-05	0.4867	0.5469	0.606	40000	160002	0.55 [0.49, 0.61]
Se1	0.9715	0.01039	2.45E-05	0.948	0.9727	0.9882	40000	160002	0.97 [0.95, 0.99]
Se2	0.9754	0.0164	4.03E-05	0.9367	0.978	0.9985	40000	160002	0.98 [0.94, 0.99]
Se3	0.9452	0.01882	4.67E-05	0.9036	0.947	0.9769	40000	160002	0.95 [0.90, 0.98]
Sp1	0.6971	0.03954	9.69E-05	0.6171	0.6978	0.772	40000	160002	0.70 [0.62, 0.77]
Sp2	0.9873	0.0125	3.24E-05	0.9538	0.9911	0.9997	40000	160002	0.99 [0.95, 0.99]
Sp3	0.9255	0.02369	5.55E-05	0.8738	0.9276	0.9658	40000	160002	0.93 [0.87, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5467	0.03051	8.90E-05	0.4868	0.5468	0.6061	60000	120002	0.55 [0.49, 0.61]
Se1	0.9715	0.01039	2.79E-05	0.948	0.9727	0.9882	60000	120002	0.97 [0.95, 0.99]
Se2	0.9754	0.01644	4.69E-05	0.9366	0.978	0.9985	60000	120002	0.98 [0.94, 0.99]
Se3	0.9452	0.01883	5.49E-05	0.9035	0.947	0.9769	60000	120002	0.95 [0.90, 0.98]
Sp1	0.697	0.03954	1.13E-04	0.617	0.6978	0.7718	60000	120002	0.70 [0.62, 0.77]
Sp2	0.9873	0.01254	3.66E-05	0.9534	0.9911	0.9997	60000	120002	0.99 [0.95, 0.99]
Sp3	0.9255	0.02373	6.29E-05	0.8737	0.9276	0.9659	60000	120002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5466	0.03053	1.08E-04	0.4866	0.5467	0.6059	80000	80002	0.55 [0.49, 0.61]
Se1	0.9715	0.01039	3.49E-05	0.948	0.9727	0.9882	80000	80002	0.97 [0.95, 0.99]
Se2	0.9754	0.01641	5.58E-05	0.9366	0.9779	0.9984	80000	80002	0.98 [0.94, 0.99]
Se3	0.9452	0.01888	6.57E-05	0.9034	0.947	0.9768	80000	80002	0.95 [0.90, 0.98]
Sp1	0.697	0.03958	1.43E-04	0.6172	0.6978	0.7719	80000	80002	0.70 [0.62, 0.77]
Sp2	0.9872	0.01257	4.42E-05	0.9534	0.9911	0.9997	80000	80002	0.99 [0.95, 0.99]
Sp3	0.9255	0.0237	7.68E-05	0.8736	0.9276	0.9657	80000	80002	0.93 [0.87, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5464	0.03047	1.57E-04	0.4864	0.5464	0.6055	100000	40002	0.55 [0.49, 0.61]
Se1	0.9715	0.01039	5.18E-05	0.9481	0.9727	0.9882	100000	40002	0.97 [0.95, 0.99]
Se2	0.9755	0.01633	7.78E-05	0.937	0.9781	0.9985	100000	40002	0.98 [0.94, 0.99]
Se3	0.9451	0.01891	9.64E-05	0.9032	0.947	0.9769	100000	40002	0.95 [0.90, 0.98]
Sp1	0.6969	0.03946	1.92E-04	0.6176	0.6976	0.7711	100000	40002	0.70 [0.62, 0.77]
Sp2	0.9872	0.01259	6.18E-05	0.9532	0.9911	0.9997	100000	40002	0.99 [0.95, 0.99]
Sp3	0.9254	0.02377	1.10E-04	0.8737	0.9277	0.9657	100000	40002	0.93 [0.87, 0.97]

Pr1 = Prevalence of disease in the population.

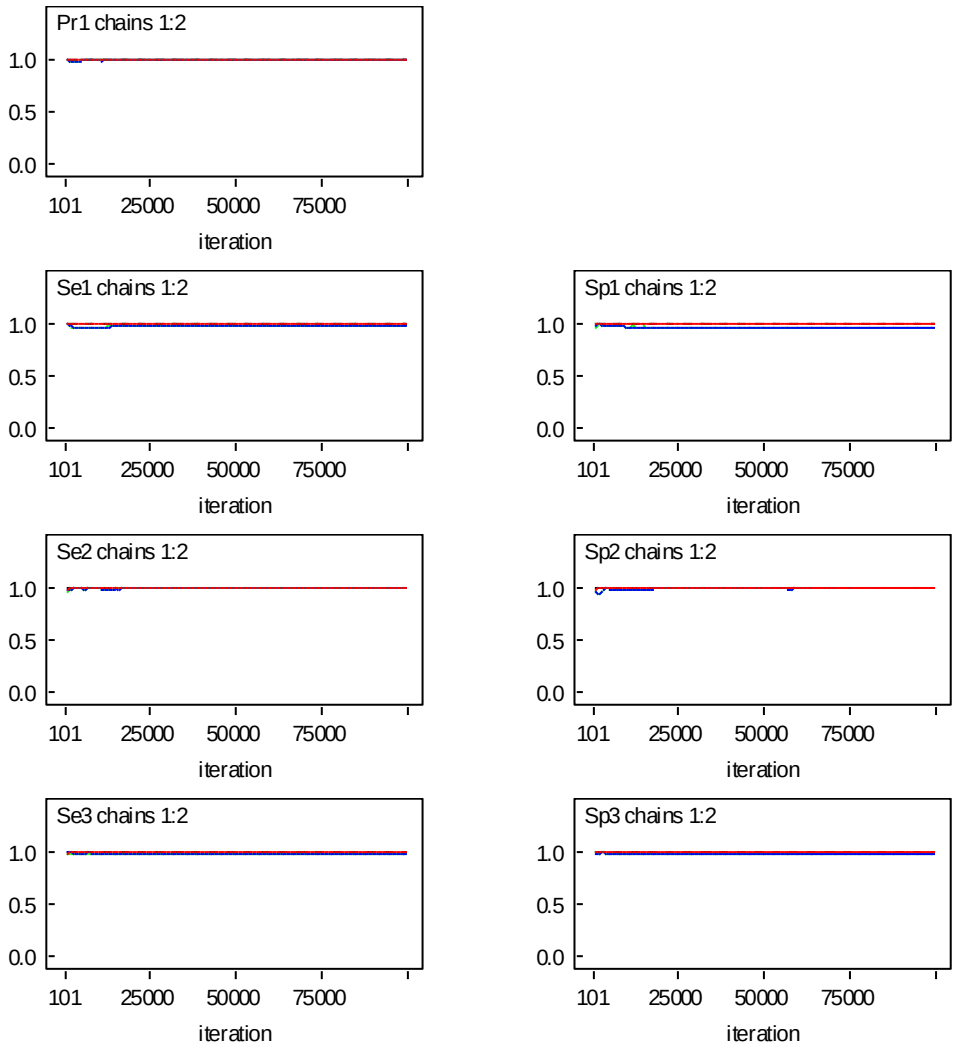
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.11.2: Plots of iterates for each variable to check for convergence for the data set for the cases where tissue pigments were absent.



Pr1 = Prevalence of disease in the population.

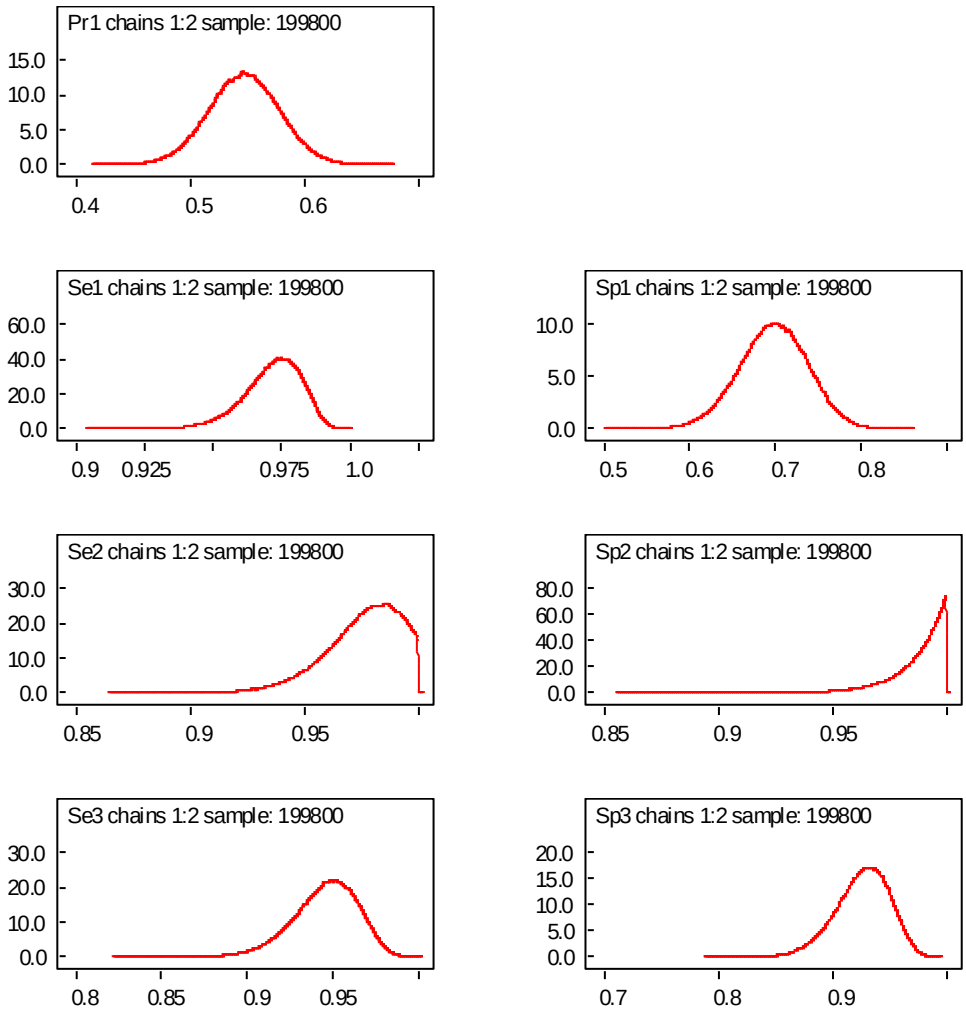
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.11.3: Kernel density estimate for the data set for the cases where tissue pigments were absent.



Pr1 = Prevalence of disease in the population.

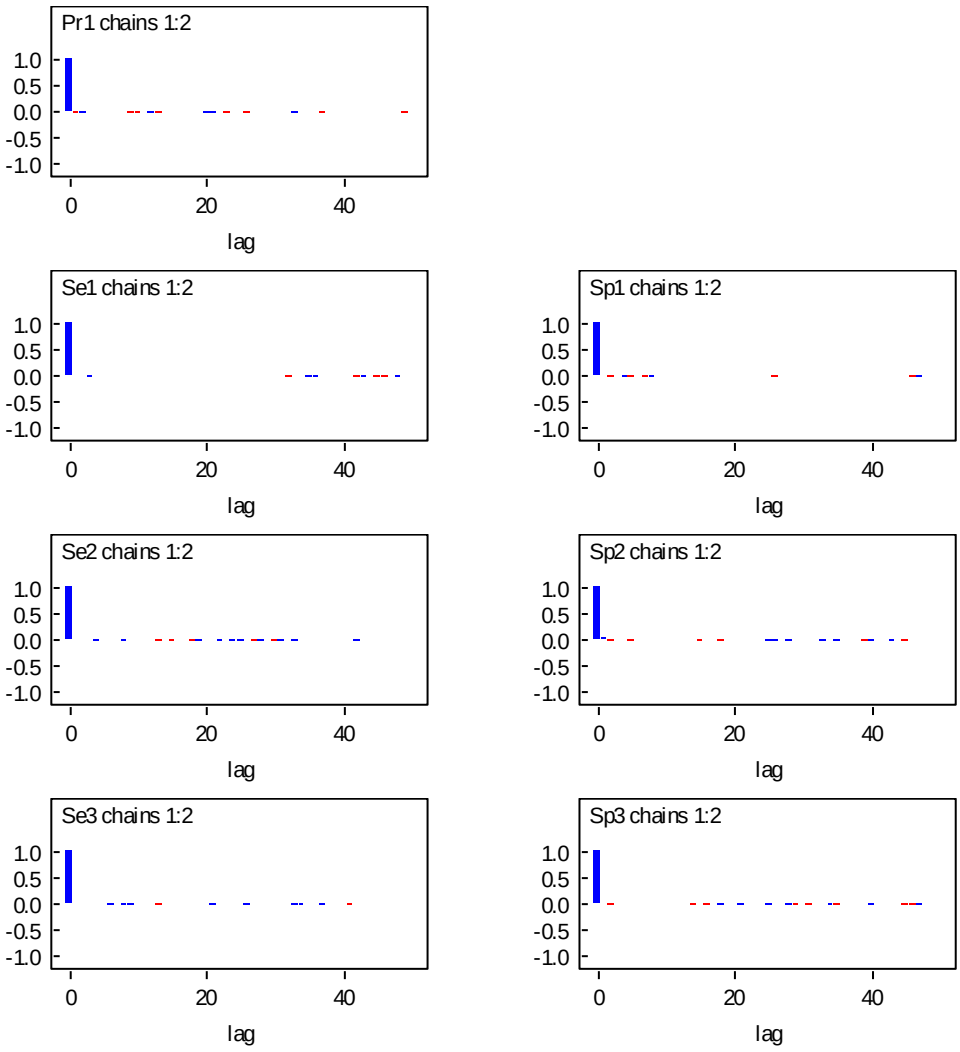
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 9.11.4: Autocorrelation plots for the data set for the cases where tissue pigments were absent.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10: Results of sensitivity analysis.

Appendix 10.1: Sensitivity analysis of the data set using an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC.

Appendix 10.1.1: Posterior values obtained for each parameter for the data set using an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5272	0.02585	1.40E-04	0.4765	0.5273	0.5779	101	39800	0.53 [0.48, 0.58]
Se1	0.9663	0.01262	6.23E-05	0.938	0.9679	0.9866	101	39800	0.97 [0.94, 0.99]
Se2	0.9744	0.01534	8.04E-05	0.9399	0.9762	0.9979	101	39800	0.98 [0.94, 0.99]
Se3	0.945	0.01599	8.10E-05	0.9101	0.9463	0.9726	101	39800	0.95 [0.91, 0.97]
Sp1	0.7079	0.03433	1.70E-04	0.6396	0.7084	0.7732	101	39800	0.71 [0.64, 0.77]
Sp2	0.9916	0.00832	4.07E-05	0.9695	0.9942	0.9998	101	39800	0.99 [0.97, 0.99]
Sp3	0.921	0.02107	1.06E-04	0.8758	0.9226	0.9577	101	39800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5272	0.02586	9.50E-05	0.4765	0.5272	0.5777	101	79800	0.53 [0.48, 0.58]
Se1	0.9664	0.01259	4.68E-05	0.9379	0.9679	0.9866	101	79800	0.97 [0.94, 0.99]
Se2	0.9744	0.01536	5.35E-05	0.9398	0.9762	0.9979	101	79800	0.98 [0.94, 0.99]
Se3	0.9449	0.01603	5.41E-05	0.9101	0.9463	0.9724	101	79800	0.95 [0.91, 0.97]
Sp1	0.7081	0.03423	1.20E-04	0.6396	0.7086	0.7732	101	79800	0.71 [0.64, 0.77]
Sp2	0.9916	0.00829	3.01E-05	0.9695	0.9942	0.9998	101	79800	0.99 [0.97, 0.99]
Sp3	0.921	0.02102	6.88E-05	0.8758	0.9225	0.9578	101	79800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5272	0.02586	7.59E-05	0.4764	0.5273	0.5778	101	119800	0.53 [0.48, 0.58]
Se1	0.9664	0.01259	3.67E-05	0.9379	0.9679	0.9866	101	119800	0.97 [0.94, 0.99]
Se2	0.9744	0.01535	4.35E-05	0.9397	0.9763	0.9979	101	119800	0.98 [0.94, 0.99]
Se3	0.9449	0.01607	4.55E-05	0.9098	0.9463	0.9725	101	119800	0.95 [0.91, 0.97]
Sp1	0.7081	0.03422	9.89E-05	0.6395	0.7086	0.7731	101	119800	0.71 [0.64, 0.77]
Sp2	0.9916	0.00827	2.52E-05	0.9694	0.9942	0.9998	101	119800	0.99 [0.97, 0.99]
Sp3	0.921	0.02101	5.93E-05	0.8758	0.9224	0.9578	101	119800	0.92 [0.88, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5272	0.02585	6.32E-05	0.4764	0.5273	0.5777	101	159800	0.53 [0.48, 0.58]
Se1	0.9664	0.0126	3.21E-05	0.9378	0.9679	0.9866	101	159800	0.97 [0.94, 0.99]
Se2	0.9744	0.01537	3.73E-05	0.9396	0.9763	0.9979	101	159800	0.98 [0.94, 0.99]
Se3	0.9449	0.01609	3.78E-05	0.9097	0.9462	0.9724	101	159800	0.95 [0.91, 0.97]
Sp1	0.7081	0.03419	8.04E-05	0.6396	0.7087	0.7729	101	159800	0.71 [0.64, 0.77]
Sp2	0.9916	0.00827	2.04E-05	0.9694	0.9942	0.9998	101	159800	0.99 [0.97, 0.99]
Sp3	0.9209	0.02102	4.86E-05	0.8758	0.9224	0.9578	101	159800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5272	0.02584	5.95E-05	0.4764	0.5273	0.5777	101	199800	0.53 [0.48, 0.58]
Se1	0.9664	0.01262	2.87E-05	0.9377	0.9679	0.9866	101	199800	0.97 [0.94, 0.99]
Se2	0.9744	0.01539	3.35E-05	0.9395	0.9763	0.9979	101	199800	0.98 [0.94, 0.99]
Se3	0.9449	0.01609	3.57E-05	0.9097	0.9462	0.9724	101	199800	0.95 [0.91, 0.97]
Sp1	0.7081	0.03424	7.57E-05	0.6395	0.7087	0.7728	101	199800	0.71 [0.64, 0.77]
Sp2	0.9916	0.00827	1.91E-05	0.9695	0.9942	0.9998	101	199800	0.99 [0.97, 0.99]
Sp3	0.921	0.02101	4.46E-05	0.8759	0.9224	0.9578	101	199800	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5273	0.02581	5.79E-05	0.4765	0.5273	0.5777	20000	200002	0.53 [0.48, 0.58]
Se1	0.9664	0.01262	2.86E-05	0.9377	0.9679	0.9866	20000	200002	0.97 [0.94, 0.99]
Se2	0.9744	0.01541	3.35E-05	0.9395	0.9763	0.9978	20000	200002	0.98 [0.94, 0.99]
Se3	0.9448	0.01612	3.63E-05	0.9096	0.9462	0.9724	20000	200002	0.95 [0.91, 0.97]
Sp1	0.7082	0.03424	7.55E-05	0.6393	0.7089	0.7728	20000	200002	0.71 [0.64, 0.77]
Sp2	0.9916	0.00827	1.99E-05	0.9694	0.9942	0.9998	20000	200002	0.99 [0.97, 0.99]
Sp3	0.921	0.021	4.56E-05	0.876	0.9224	0.9578	20000	200002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5273	0.0258	6.46E-05	0.4766	0.5274	0.5777	40000	160002	0.53 [0.48, 0.58]
Se1	0.9664	0.01263	3.06E-05	0.9376	0.9679	0.9866	40000	160002	0.97 [0.94, 0.99]
Se2	0.9744	0.01542	3.74E-05	0.9394	0.9763	0.9979	40000	160002	0.98 [0.94, 0.99]
Se3	0.9448	0.01613	3.98E-05	0.9095	0.9462	0.9725	40000	160002	0.95 [0.91, 0.97]
Sp1	0.7082	0.03426	8.57E-05	0.6392	0.7089	0.7726	40000	160002	0.71 [0.64, 0.77]
Sp2	0.9917	0.00827	2.24E-05	0.9694	0.9942	0.9998	40000	160002	0.99 [0.97, 0.99]
Sp3	0.921	0.02101	5.03E-05	0.876	0.9224	0.9578	40000	160002	0.92 [0.88, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5273	0.02577	7.51E-05	0.4766	0.5274	0.5776	60000	120002	0.53 [0.48, 0.58]
Se1	0.9664	0.01264	3.60E-05	0.9376	0.9679	0.9867	60000	120002	0.97 [0.94, 0.99]
Se2	0.9744	0.01544	4.36E-05	0.9393	0.9763	0.9979	60000	120002	0.98 [0.94, 0.99]
Se3	0.9448	0.01613	4.61E-05	0.9095	0.9462	0.9725	60000	120002	0.95 [0.91, 0.97]
Sp1	0.7082	0.03429	9.65E-05	0.6392	0.709	0.7725	60000	120002	0.71 [0.64, 0.77]
Sp2	0.9917	0.00828	2.62E-05	0.9694	0.9942	0.9998	60000	120002	0.99 [0.97, 0.99]
Sp3	0.921	0.02101	6.15E-05	0.876	0.9224	0.9578	60000	120002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5274	0.02575	8.89E-05	0.4767	0.5274	0.5777	80000	80002	0.53 [0.48, 0.58]
Se1	0.9664	0.01266	4.47E-05	0.9376	0.9679	0.9867	80000	80002	0.97 [0.94, 0.99]
Se2	0.9744	0.01545	5.40E-05	0.9393	0.9763	0.9978	80000	80002	0.98 [0.94, 0.99]
Se3	0.9448	0.01612	5.76E-05	0.9096	0.9461	0.9725	80000	80002	0.95 [0.91, 0.97]
Sp1	0.7082	0.03437	1.21E-04	0.6388	0.7089	0.7727	80000	80002	0.71 [0.64, 0.77]
Sp2	0.9917	0.00829	3.31E-05	0.9694	0.9942	0.9998	80000	80002	0.99 [0.97, 0.99]
Sp3	0.921	0.021	7.46E-05	0.8762	0.9225	0.9578	80000	80002	0.92 [0.88, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5275	0.02571	1.25E-04	0.4772	0.5276	0.5777	100000	40002	0.53 [0.48, 0.58]
Se1	0.9665	0.01263	6.25E-05	0.9377	0.9679	0.9868	100000	40002	0.97 [0.94, 0.99]
Se2	0.9744	0.01542	7.39E-05	0.9397	0.9764	0.9979	100000	40002	0.98 [0.94, 0.99]
Se3	0.9448	0.01611	8.16E-05	0.9097	0.9461	0.9727	100000	40002	0.95 [0.91, 0.97]
Sp1	0.7083	0.0343	1.74E-04	0.6386	0.7091	0.7729	100000	40002	0.71 [0.64, 0.77]
Sp2	0.9917	0.00833	4.51E-05	0.9692	0.9942	0.9998	100000	40002	0.99 [0.97, 0.99]
Sp3	0.9209	0.02106	1.05E-04	0.8761	0.9224	0.9578	100000	40002	0.92 [0.88, 0.96]

Pr1 = Prevalence of disease in the population.

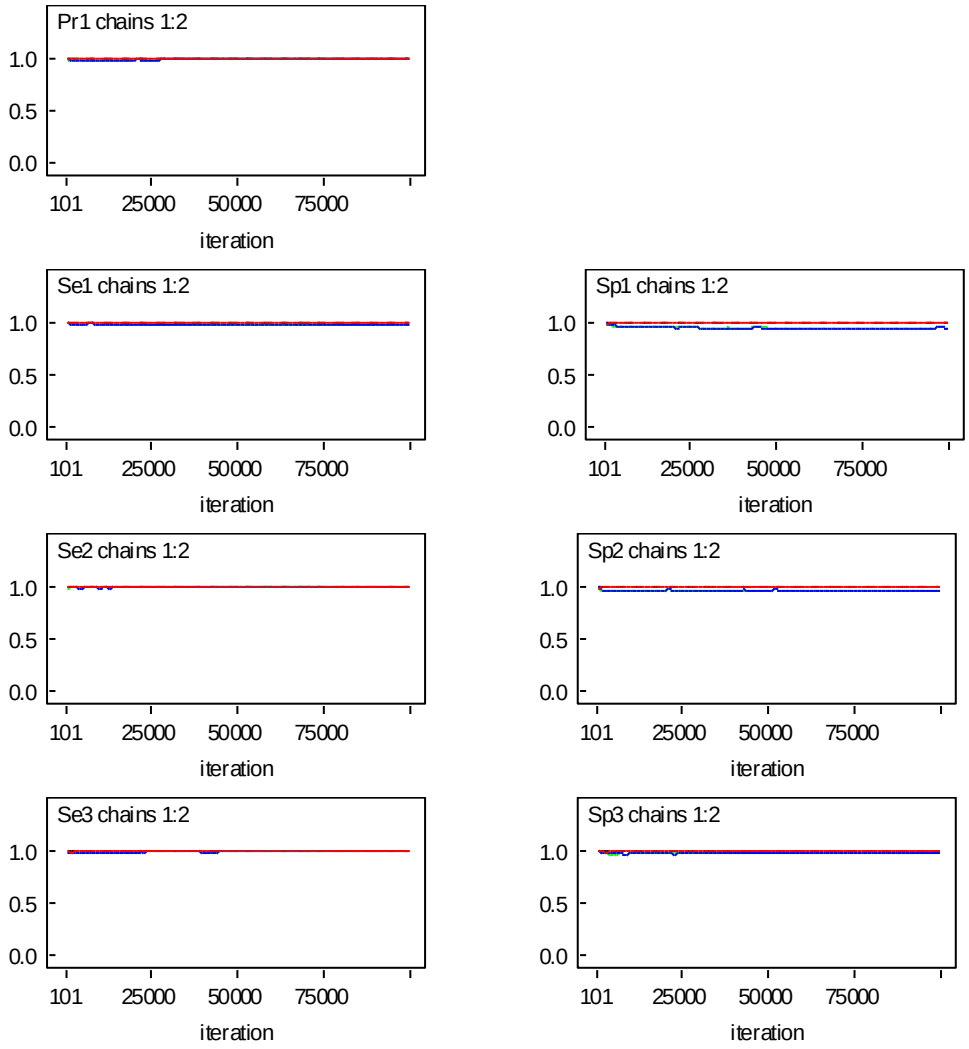
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.1.2: Plots of iterates for each variable to check for convergence for the data set using an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC.



Pr1 = Prevalence of disease in the population.

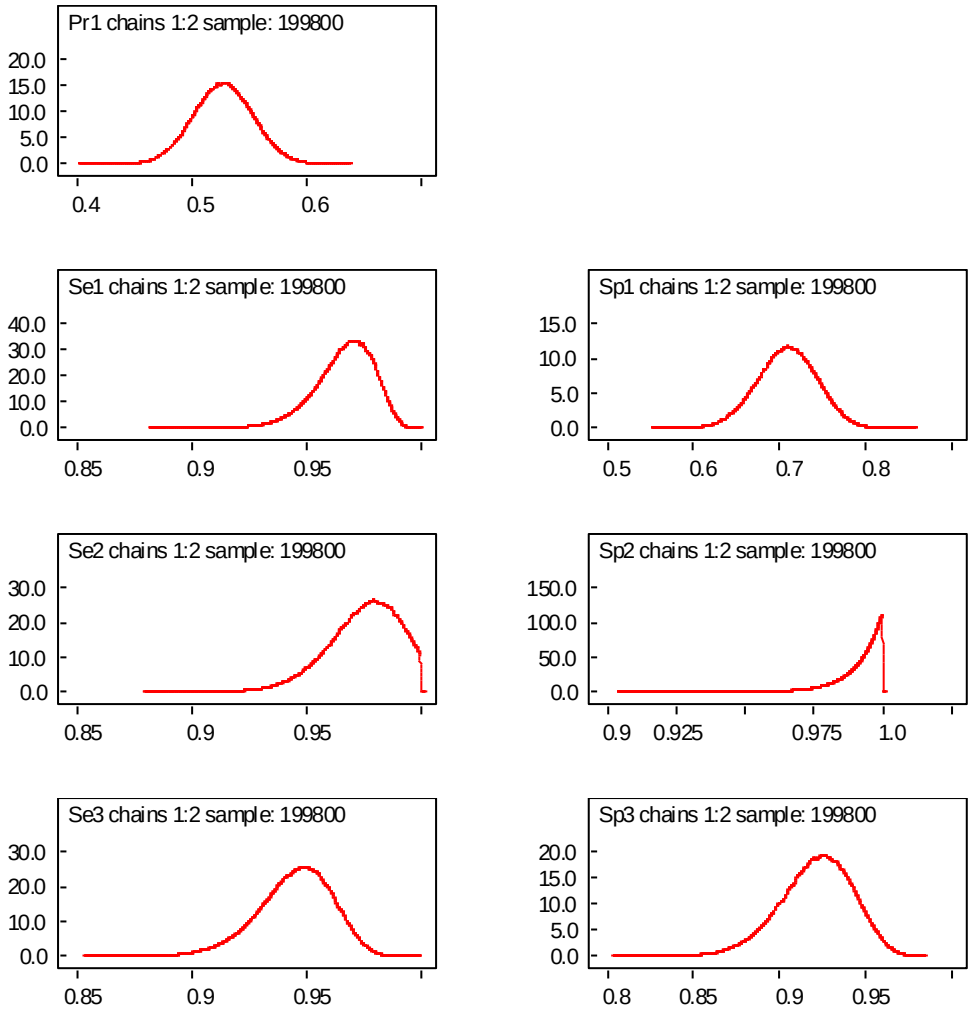
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.1.3: Kernel density estimate for the data set using an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC.



Pr1 = Prevalence of disease in the population.

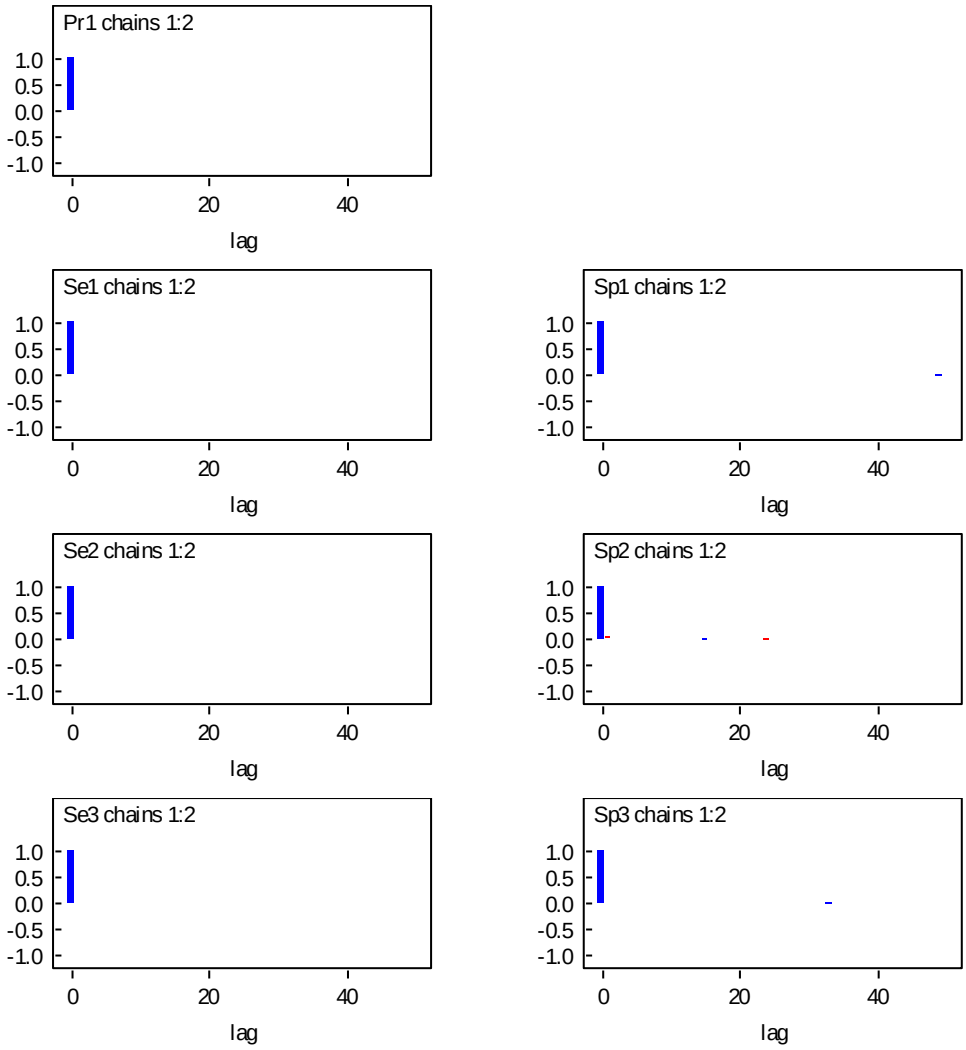
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.1.4: Autocorrelation plots for the data set using an informative prior for histopathology and uninformative priors for prevalence, rRT-PCR and IHC.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.2: Sensitivity analysis of the data set using an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC.

Appendix 10.2.1: Posterior values obtained for each parameter for the data set using an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5283	0.02576	1.29E-04	0.4775	0.5283	0.5787	101	39800	0.53 [0.48, 0.58]
Se1	0.9732	0.0092	4.47E-05	0.9523	0.9741	0.9882	101	39800	0.97 [0.95, 0.99]
Se2	0.9723	0.01558	7.70E-05	0.9375	0.974	0.9971	101	39800	0.97 [0.94, 0.99]
Se3	0.9438	0.0164	8.01E-05	0.9081	0.9451	0.9719	101	39800	0.95 [0.91, 0.97]
Sp1	0.7176	0.03258	1.51E-04	0.6525	0.7183	0.7795	101	39800	0.72 [0.65, 0.78]
Sp2	0.9917	0.00817	4.44E-05	0.9696	0.9942	0.9998	101	39800	0.99 [0.97, 0.99]
Sp3	0.9281	0.02168	1.13E-04	0.8808	0.9299	0.9653	101	39800	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5284	0.02574	9.34E-05	0.4778	0.5284	0.5788	101	79800	0.53 [0.48, 0.58]
Se1	0.9731	0.00921	3.37E-05	0.9524	0.9741	0.9882	101	79800	0.97 [0.95, 0.99]
Se2	0.9723	0.01566	5.13E-05	0.9373	0.974	0.9971	101	79800	0.97 [0.94, 0.99]
Se3	0.9437	0.01639	5.70E-05	0.908	0.9452	0.9718	101	79800	0.95 [0.91, 0.97]
Sp1	0.7175	0.0327	1.11E-04	0.6521	0.7182	0.7797	101	79800	0.72 [0.65, 0.78]
Sp2	0.9918	0.00818	2.92E-05	0.9697	0.9942	0.9998	101	79800	0.99 [0.97, 0.99]
Sp3	0.9281	0.0216	8.06E-05	0.8812	0.9299	0.9652	101	79800	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5285	0.02574	7.84E-05	0.4779	0.5285	0.5789	101	119800	0.53 [0.48, 0.58]
Se1	0.9732	0.00919	2.57E-05	0.9525	0.9741	0.9882	101	119800	0.97 [0.95, 0.99]
Se2	0.9723	0.01566	4.28E-05	0.9373	0.974	0.9971	101	119800	0.97 [0.94, 0.99]
Se3	0.9436	0.01644	4.85E-05	0.9079	0.9451	0.9718	101	119800	0.95 [0.91, 0.97]
Sp1	0.7176	0.03267	9.58E-05	0.6521	0.7183	0.7797	101	119800	0.72 [0.65, 0.78]
Sp2	0.9918	0.00815	2.37E-05	0.9699	0.9943	0.9998	101	119800	0.99 [0.97, 0.99]
Sp3	0.9281	0.02162	6.44E-05	0.8812	0.9298	0.9652	101	119800	0.93 [0.88, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5286	0.02578	6.70E-05	0.478	0.5286	0.5791	101	159800	0.53 [0.48, 0.58]
Se1	0.9732	0.00918	2.35E-05	0.9526	0.9742	0.9882	101	159800	0.97 [0.95, 0.99]
Se2	0.9722	0.01568	3.83E-05	0.9372	0.974	0.9971	101	159800	0.97 [0.94, 0.99]
Se3	0.9436	0.01645	4.13E-05	0.9077	0.945	0.9717	101	159800	0.95 [0.91, 0.97]
Sp1	0.7176	0.03268	8.29E-05	0.652	0.7183	0.7797	101	159800	0.72 [0.65, 0.78]
Sp2	0.9918	0.00816	2.12E-05	0.9697	0.9943	0.9998	101	159800	0.99 [0.97, 0.99]
Sp3	0.9281	0.02161	5.62E-05	0.8812	0.9299	0.9652	101	159800	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5285	0.02577	6.00E-05	0.4778	0.5285	0.579	101	199800	0.53 [0.48, 0.58]
Se1	0.9732	0.00919	2.09E-05	0.9525	0.9742	0.9882	101	199800	0.97 [0.95, 0.99]
Se2	0.9722	0.01569	3.46E-05	0.9371	0.974	0.9971	101	199800	0.97 [0.94, 0.99]
Se3	0.9436	0.01644	3.59E-05	0.9077	0.945	0.9717	101	199800	0.95 [0.91, 0.97]
Sp1	0.7177	0.03268	7.38E-05	0.6519	0.7184	0.7798	101	199800	0.72 [0.65, 0.78]
Sp2	0.9918	0.00816	1.90E-05	0.9697	0.9943	0.9998	101	199800	0.99 [0.97, 0.99]
Sp3	0.9281	0.02161	4.74E-05	0.8812	0.9299	0.9652	101	199800	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5285	0.02582	5.96E-05	0.4778	0.5286	0.579	20000	200002	0.53 [0.48, 0.58]
Se1	0.9732	0.00919	2.09E-05	0.9526	0.9741	0.9882	20000	200002	0.97 [0.95, 0.99]
Se2	0.9722	0.01572	3.63E-05	0.937	0.9739	0.9971	20000	200002	0.97 [0.94, 0.99]
Se3	0.9436	0.01645	3.71E-05	0.9077	0.945	0.9717	20000	200002	0.95 [0.91, 0.97]
Sp1	0.7177	0.03271	7.45E-05	0.652	0.7184	0.7798	20000	200002	0.72 [0.65, 0.78]
Sp2	0.9918	0.00816	1.84E-05	0.9698	0.9943	0.9998	20000	200002	0.99 [0.97, 0.99]
Sp3	0.9281	0.02159	4.63E-05	0.8812	0.9299	0.9652	20000	200002	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5285	0.02585	6.73E-05	0.4777	0.5286	0.5791	40000	160002	0.53 [0.48, 0.58]
Se1	0.9732	0.00918	2.30E-05	0.9526	0.9742	0.9882	40000	160002	0.97 [0.95, 0.99]
Se2	0.9722	0.01572	4.02E-05	0.937	0.9739	0.9971	40000	160002	0.97 [0.94, 0.99]
Se3	0.9435	0.01647	3.97E-05	0.9076	0.945	0.9717	40000	160002	0.95 [0.91, 0.97]
Sp1	0.7178	0.03268	8.43E-05	0.6521	0.7185	0.7799	40000	160002	0.72 [0.65, 0.78]
Sp2	0.9918	0.00816	2.00E-05	0.9698	0.9943	0.9998	40000	160002	0.99 [0.97, 0.99]
Sp3	0.9282	0.0216	5.33E-05	0.8811	0.9299	0.9653	40000	160002	0.93 [0.88, 0.97]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5284	0.02588	7.43E-05	0.4775	0.5285	0.5791	60000	120002	0.53 [0.48, 0.58]
Se1	0.9732	0.00918	2.60E-05	0.9525	0.9742	0.9881	60000	120002	0.97 [0.95, 0.99]
Se2	0.9722	0.01574	4.47E-05	0.9369	0.9739	0.9972	60000	120002	0.97 [0.94, 0.99]
Se3	0.9436	0.01645	4.77E-05	0.9076	0.945	0.9717	60000	120002	0.95 [0.91, 0.97]
Sp1	0.7179	0.03271	9.73E-05	0.6521	0.7185	0.78	60000	120002	0.72 [0.65, 0.78]
Sp2	0.9918	0.00818	2.35E-05	0.9697	0.9943	0.9998	60000	120002	0.99 [0.97, 0.99]
Sp3	0.9282	0.02159	6.14E-05	0.8811	0.9299	0.9653	60000	120002	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5283	0.02588	8.27E-05	0.4774	0.5283	0.5788	80000	80002	0.53 [0.48, 0.58]
Se1	0.9731	0.00921	3.24E-05	0.9525	0.9741	0.9881	80000	80002	0.97 [0.95, 0.99]
Se2	0.9722	0.01574	5.77E-05	0.9369	0.9739	0.9972	80000	80002	0.97 [0.94, 0.99]
Se3	0.9436	0.01643	6.12E-05	0.9077	0.9451	0.9718	80000	80002	0.95 [0.91, 0.97]
Sp1	0.7179	0.03271	1.21E-04	0.6522	0.7186	0.7801	80000	80002	0.72 [0.65, 0.78]
Sp2	0.9918	0.00817	2.85E-05	0.9698	0.9943	0.9998	80000	80002	0.99 [0.97, 0.99]
Sp3	0.9281	0.02159	7.25E-05	0.881	0.9299	0.9653	80000	80002	0.93 [0.88, 0.97]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5283	0.02602	1.25E-04	0.4772	0.5284	0.5791	100000	40002	0.53 [0.48, 0.58]
Se1	0.9731	0.0092	4.48E-05	0.9525	0.9741	0.9881	100000	40002	0.97 [0.95, 0.99]
Se2	0.9721	0.01577	7.94E-05	0.9368	0.974	0.9972	100000	40002	0.97 [0.94, 0.99]
Se3	0.9437	0.01646	8.97E-05	0.9077	0.9451	0.9719	100000	40002	0.95 [0.91, 0.97]
Sp1	0.718	0.03273	1.67E-04	0.6528	0.7186	0.7798	100000	40002	0.72 [0.65, 0.78]
Sp2	0.9917	0.00816	3.95E-05	0.97	0.9942	0.9998	100000	40002	0.99 [0.97, 0.99]
Sp3	0.9282	0.02157	1.07E-04	0.8809	0.9299	0.9653	100000	40002	0.93 [0.88, 0.97]

Pr1 = Prevalence of disease in the population.

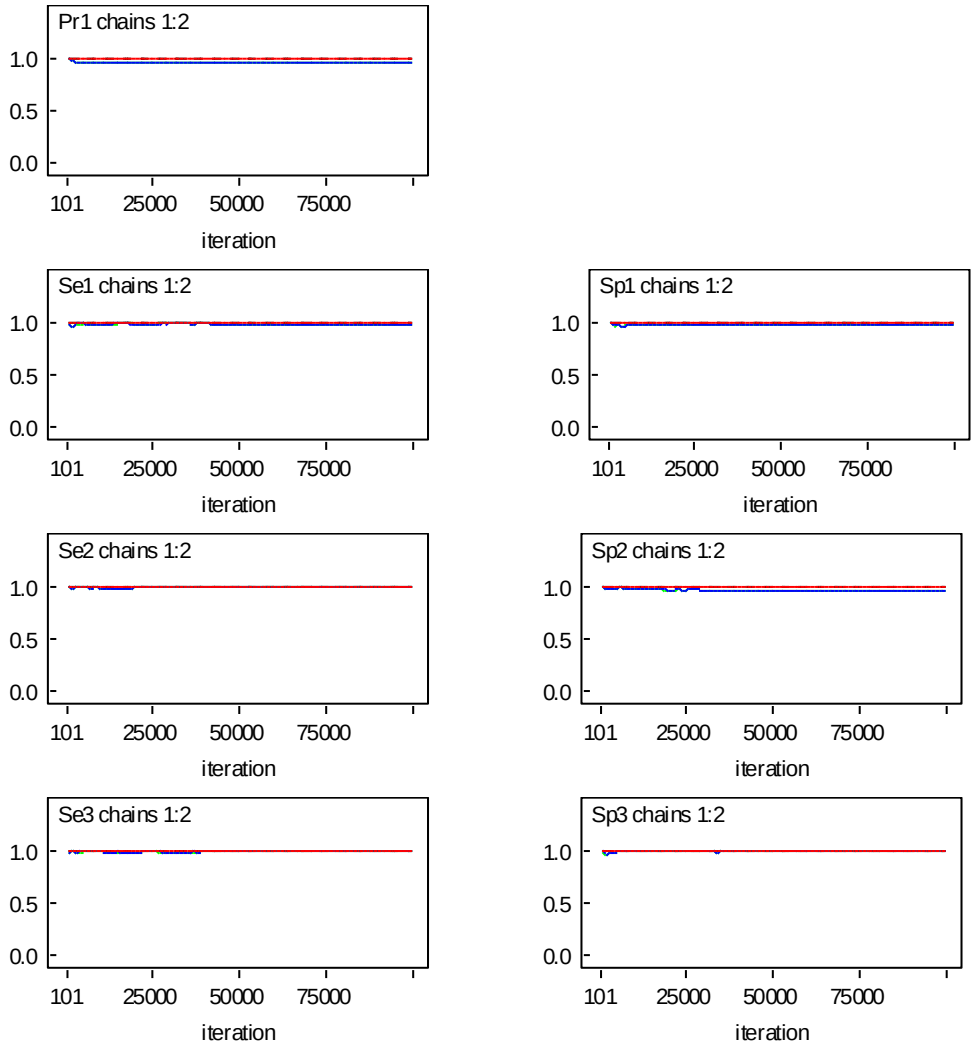
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.2.2: Plots of iterates for each variable to check for convergence for the data set using an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

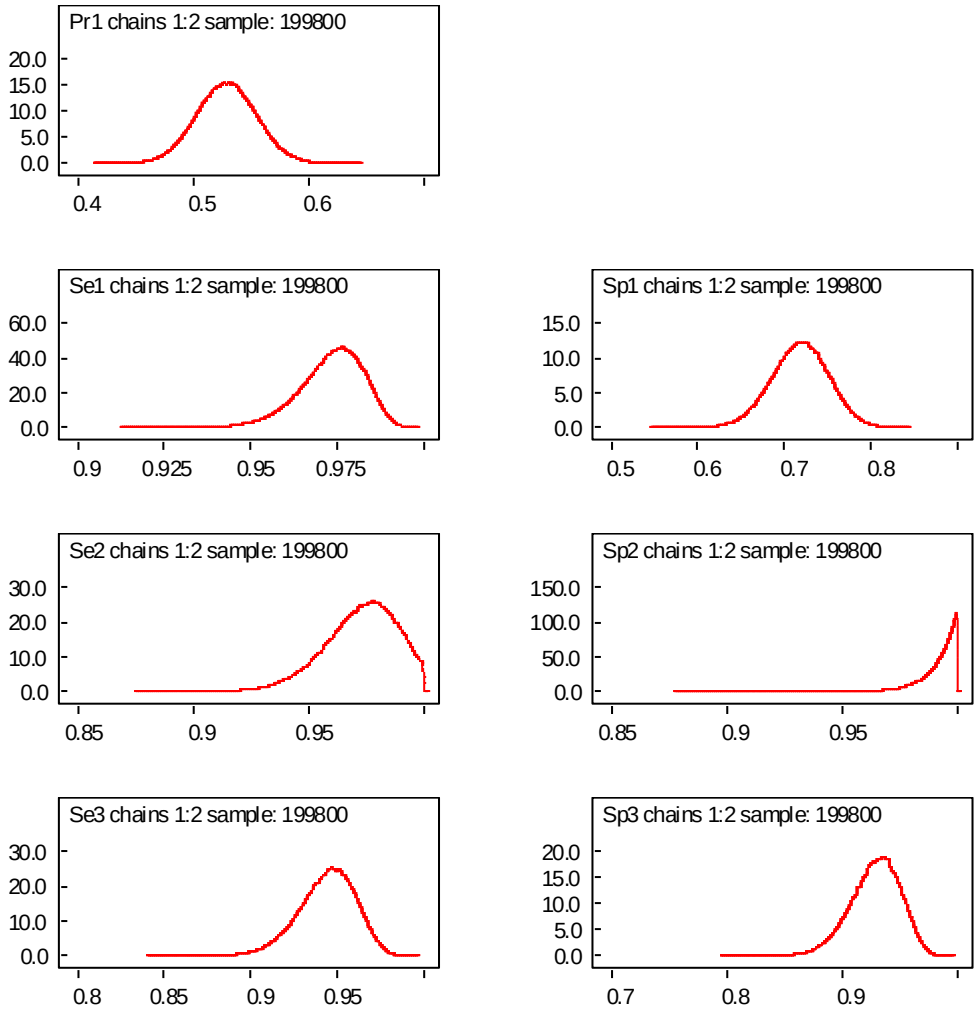
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.2.3: Kernel density estimate for the data set using an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

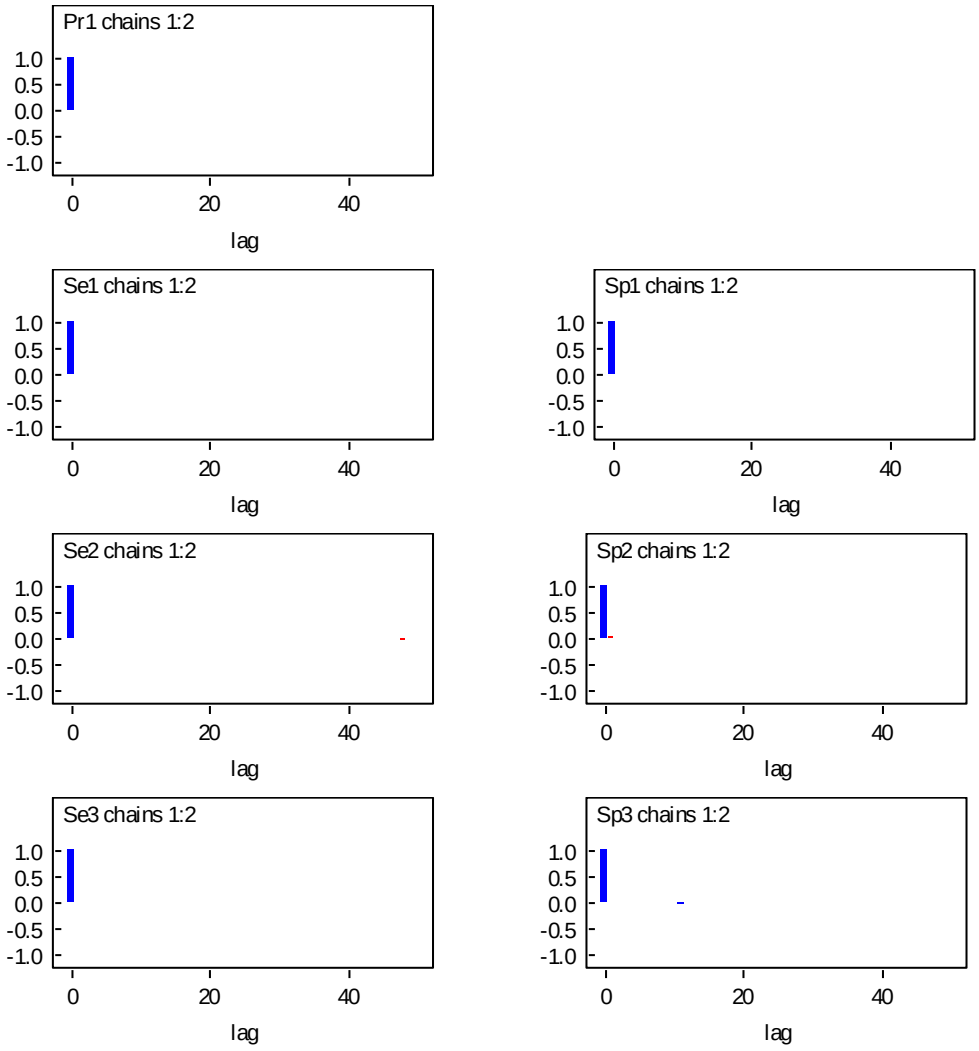
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.2.4: Autocorrelation plots for the data set using an informative prior for rRT-PCR and uninformative priors for prevalence, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.3: Sensitivity analysis of the data set using uninformative priors for prevalence, rRT-PCR, histopathology, and IHC.

Appendix 10.3.1: Posterior values obtained for each parameter for the data set using uninformative priors for prevalence, rRT-PCR, histopathology, and IHC.

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5004	0.03833	1.70E-03	0.4298	0.5005	0.5706	101	39800	0.50 [0.43, 0.57]
Se1	0.6282	0.339	2.02E-02	0.2353	0.8959	0.9842	101	39800	0.90 [0.24, 0.98]
Se2	0.4903	0.4822	2.88E-02	4.20E-04	0.8723	0.9952	101	39800	0.87 [0.00, 0.99]
Se3	0.5077	0.4365	2.61E-02	0.03917	0.8632	0.968	101	39800	0.86 [0.04, 0.97]
Sp1	0.3714	0.3388	2.02E-02	0.01555	0.5766	0.7645	101	39800	0.58 [0.02, 0.76]
Sp2	0.5097	0.4822	2.88E-02	0.004921	0.8977	0.9996	101	39800	0.90 [0.00, 0.99]
Sp3	0.4922	0.4364	2.60E-02	0.03164	0.8346	0.9607	101	39800	0.83 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5001	0.03834	1.42E-03	0.4297	0.5002	0.5704	101	79800	0.50 [0.43, 0.57]
Se1	0.6283	0.3389	1.69E-02	0.2353	0.8899	0.9842	101	79800	0.89 [0.24, 0.98]
Se2	0.4903	0.4822	2.41E-02	4.20E-04	0.8602	0.9952	101	79800	0.86 [0.00, 0.99]
Se3	0.5077	0.4364	2.18E-02	0.03909	0.8553	0.9681	101	79800	0.86 [0.04, 0.97]
Sp1	0.3715	0.3387	1.69E-02	0.01565	0.5766	0.7649	101	79800	0.58 [0.02, 0.76]
Sp2	0.5097	0.4823	2.41E-02	0.004837	0.8977	0.9996	101	79800	0.90 [0.00, 0.99]
Sp3	0.4921	0.4363	2.18E-02	0.03169	0.8129	0.9606	101	79800	0.81 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	**Posterior value**
Pr1	0.5000	0.03833	1.28E-03	0.4296	0.5000	0.5705	101	119800	0.50 [0.43, 0.57]
Se1	0.6283	0.3389	1.53E-02	0.2353	0.8899	0.9843	101	119800	0.89 [0.24, 0.98]
Se2	0.4903	0.4822	2.18E-02	4.25E-04	0.8602	0.9951	101	119800	0.86 [0.00, 0.99]
Se3	0.5078	0.4364	1.97E-02	0.03944	0.8553	0.9681	101	119800	0.86 [0.04, 0.97]
Sp1	0.3716	0.3388	1.53E-02	0.01571	0.5555	0.7649	101	119800	0.56 [0.02, 0.76]
Sp2	0.5097	0.4823	2.18E-02	0.004735	0.8784	0.9996	101	119800	0.88 [0.00, 0.99]
Sp3	0.4921	0.4363	1.97E-02	0.03172	0.8129	0.9606	101	119800	0.81 [0.03, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5000	0.03831	1.19E-03	0.4296	0.5000	0.5706	101	159800	0.50 [0.43, 0.57]
Se1	0.6284	0.3389	1.42E-02	0.2354	0.8889	0.9843	101	159800	0.89 [0.24, 0.98]
Se2	0.4903	0.4822	2.03E-02	4.31E-04	0.8602	0.9951	101	159800	0.86 [0.00, 0.99]
Se3	0.5078	0.4363	1.83E-02	0.0393	0.8553	0.9681	101	159800	0.86 [0.04, 0.97]
Sp1	0.3717	0.3389	1.42E-02	0.01574	0.5555	0.7649	101	159800	0.56 [0.02, 0.76]
Sp2	0.5097	0.4823	2.03E-02	0.004741	0.8784	0.9996	101	159800	0.88 [0.00, 0.99]
Sp3	0.4921	0.4363	1.83E-02	0.0318	0.8129	0.9606	101	159800	0.81 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.5000	0.03833	1.13E-03	0.4294	0.5000	0.5706	101	199800	0.50 [0.43, 0.57]
Se1	0.6284	0.3389	1.35E-02	0.2353	0.8845	0.9842	101	199800	0.88 [0.24, 0.98]
Se2	0.4903	0.4823	1.92E-02	4.30E-04	0.8602	0.9952	101	199800	0.86 [0.00, 0.99]
Se3	0.5078	0.4363	1.74E-02	0.03931	0.8553	0.9681	101	199800	0.86 [0.04, 0.97]
Sp1	0.3717	0.339	1.35E-02	0.01571	0.5555	0.7649	101	199800	0.56 [0.02, 0.76]
Sp2	0.5096	0.4823	1.92E-02	0.004743	0.8784	0.9996	101	199800	0.88 [0.00, 0.99]
Sp3	0.4921	0.4362	1.74E-02	0.03179	0.8129	0.9606	101	199800	0.81 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4999	0.03833	1.13E-03	0.4292	0.4999	0.5706	20000	200002	0.50 [0.43, 0.57]
Se1	0.6284	0.3388	1.35E-02	0.2353	0.8845	0.9842	20000	200002	0.88 [0.24, 0.98]
Se2	0.4904	0.4823	1.92E-02	4.31E-04	0.8602	0.9952	20000	200002	0.86 [0.00, 0.99]
Se3	0.5078	0.4363	1.74E-02	0.03928	0.8533	0.968	20000	200002	0.85 [0.04, 0.97]
Sp1	0.3717	0.3389	1.35E-02	0.01577	0.5356	0.7649	20000	200002	0.54 [0.02, 0.76]
Sp2	0.5096	0.4823	1.92E-02	0.004712	0.8784	0.9996	20000	200002	0.88 [0.00, 0.99]
Sp3	0.4921	0.4362	1.74E-02	0.0318	0.8129	0.9605	20000	200002	0.81 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4999	0.03832	1.19E-03	0.4291	0.4998	0.5707	40000	160002	0.50 [0.43, 0.57]
Se1	0.6284	0.3388	1.42E-02	0.2353	0.8845	0.9841	40000	160002	0.88 [0.24, 0.98]
Se2	0.4904	0.4823	2.03E-02	4.33E-04	0.8768	0.9952	40000	160002	0.88 [0.00, 0.99]
Se3	0.5078	0.4362	1.83E-02	0.03935	0.8533	0.968	40000	160002	0.85 [0.04, 0.97]
Sp1	0.3717	0.339	1.42E-02	0.01577	0.5356	0.7647	40000	160002	0.54 [0.02, 0.76]
Sp2	0.5097	0.4823	2.03E-02	0.004701	0.8784	0.9996	40000	160002	0.88 [0.00, 0.99]
Sp3	0.4921	0.4362	1.83E-02	0.03181	0.8167	0.9605	40000	160002	0.82 [0.03, 0.96]

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node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4999	0.03833	1.28E-03	0.4290	0.4999	0.5706	60000	120002	0.50 [0.43, 0.57]
Se1	0.6284	0.3388	1.53E-02	0.2353	0.8845	0.9841	60000	120002	0.88 [0.24, 0.98]
Se2	0.4904	0.4823	2.18E-02	4.32E-04	0.8807	0.9952	60000	120002	0.88 [0.00, 0.99]
Se3	0.5078	0.4362	1.97E-02	0.03914	0.8533	0.9679	60000	120002	0.85 [0.04, 0.97]
Sp1	0.3717	0.339	1.53E-02	0.01575	0.5356	0.7647	60000	120002	0.54 [0.02, 0.76]
Sp2	0.5097	0.4824	2.18E-02	0.004746	0.9184	0.9996	60000	120002	0.92 [0.00, 0.99]
Sp3	0.4921	0.4362	1.97E-02	0.03181	0.8167	0.9605	60000	120002	0.82 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4998	0.03836	1.43E-03	0.4287	0.4999	0.5706	80000	80002	0.50 [0.43, 0.57]
Se1	0.6284	0.3388	1.69E-02	0.2351	0.8845	0.984	80000	80002	0.88 [0.24, 0.98]
Se2	0.4904	0.4824	2.42E-02	4.26E-04	0.8807	0.9953	80000	80002	0.88 [0.00, 0.99]
Se3	0.5078	0.4363	2.19E-02	0.03921	0.8533	0.9679	80000	80002	0.85 [0.04, 0.97]
Sp1	0.3715	0.3389	1.69E-02	0.01573	0.5356	0.7647	80000	80002	0.54 [0.02, 0.76]
Sp2	0.5097	0.4824	2.42E-02	0.004746	0.9188	0.9996	80000	80002	0.92 [0.00, 0.99]
Sp3	0.4921	0.4362	2.18E-02	0.03174	0.8167	0.9604	80000	80002	0.82 [0.03, 0.96]

node	mean	sd	MC error	2.50%	median	97.50%	start	sample	Posterior value
Pr1	0.4997	0.03833	1.70E-03	0.4288	0.4998	0.5704	100000	40002	0.50 [0.43, 0.57]
Se1	0.6284	0.3387	2.01E-02	0.2354	0.8951	0.984	100000	40002	0.90 [0.24, 0.98]
Se2	0.4904	0.4824	2.87E-02	4.26E-04	0.8952	0.9952	100000	40002	0.90 [0.00, 0.99]
Se3	0.5078	0.4364	2.60E-02	0.03904	0.8533	0.9678	100000	40002	0.85 [0.04, 0.97]
Sp1	0.3713	0.3387	2.01E-02	0.01584	0.5356	0.7644	100000	40002	0.54 [0.02, 0.76]
Sp2	0.5098	0.4824	2.87E-02	0.004746	0.9206	0.9996	100000	40002	0.92 [0.00, 0.99]
Sp3	0.4922	0.4362	2.60E-02	0.03166	0.8185	0.9601	100000	40002	0.82 [0.03, 0.96]

Pr1 = Prevalence of disease in the population.

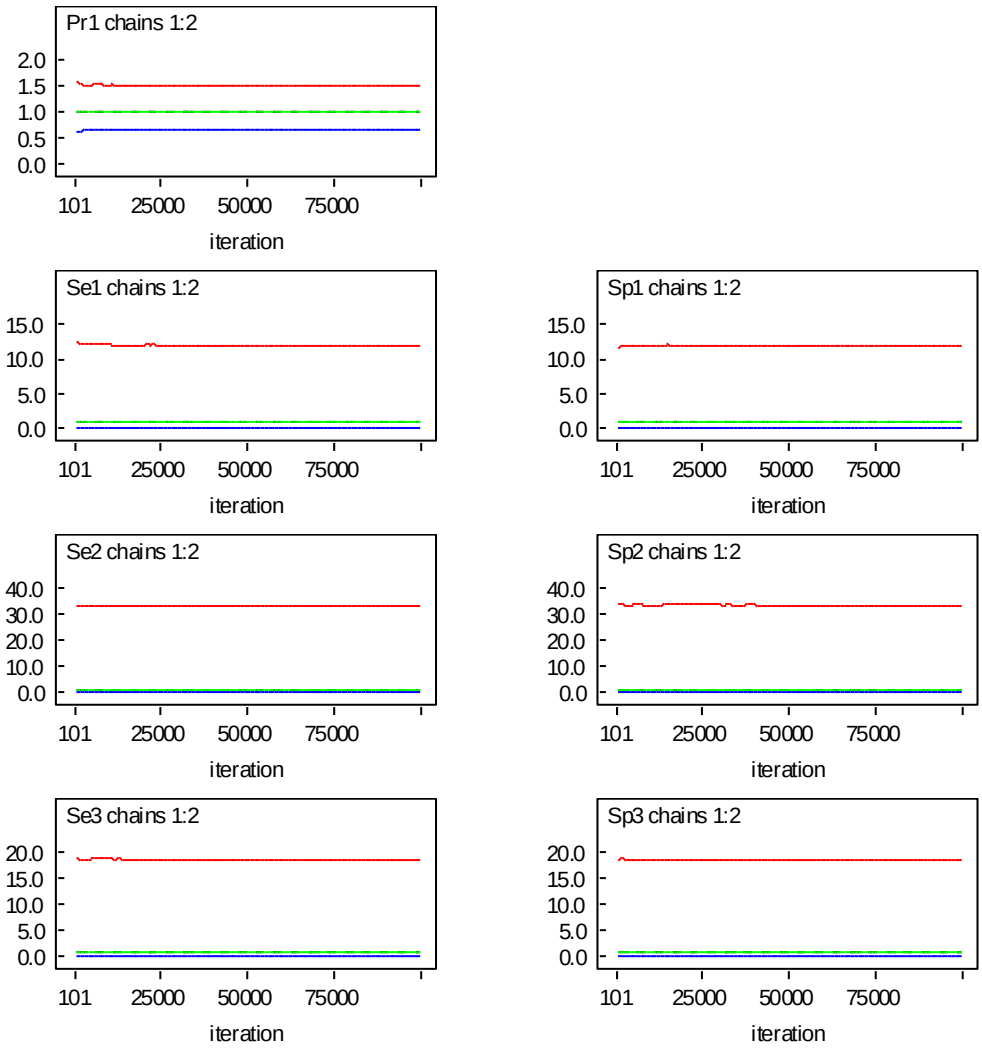
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.3.2: Plots of iterates for each variable to check for convergence for the data set using uninformative priors for prevalence, rRT-PCR, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

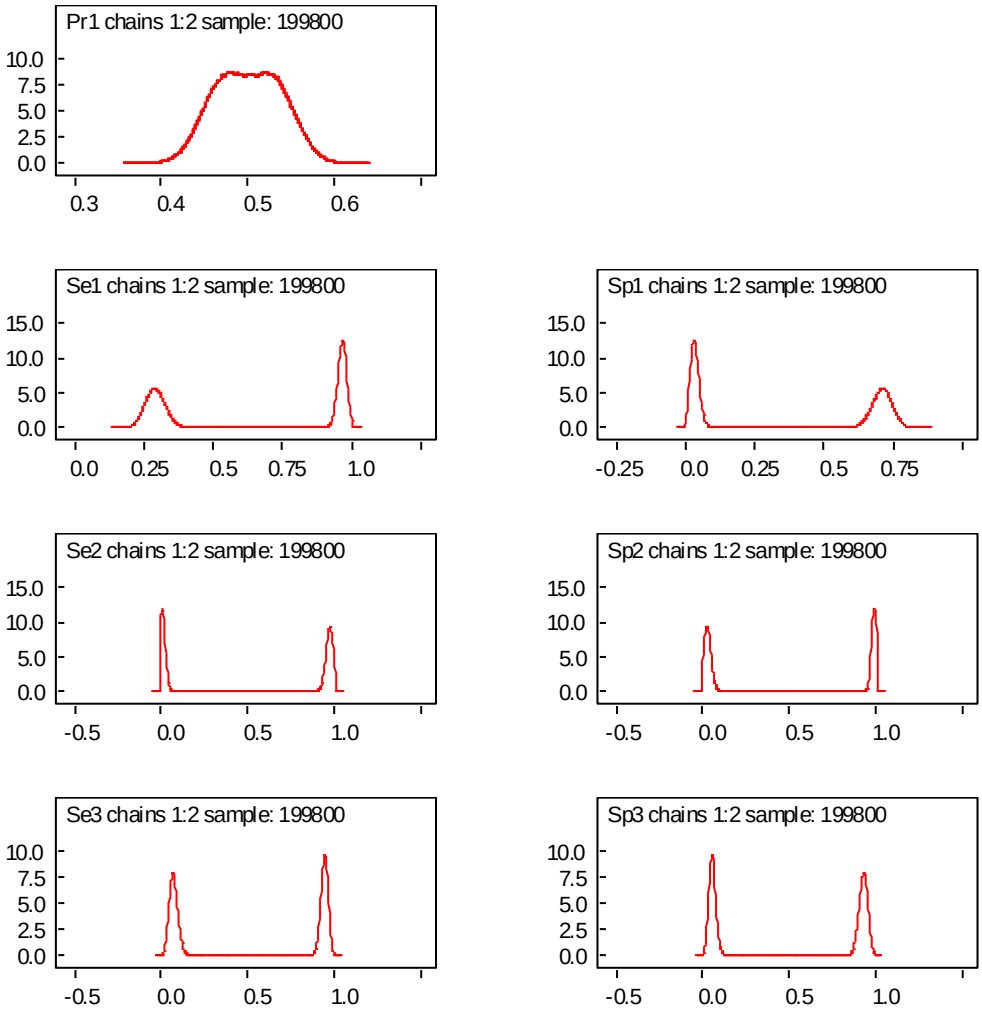
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.3.3: Kernel density estimate for the data set using uninformative priors for prevalence, rRT-PCR, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

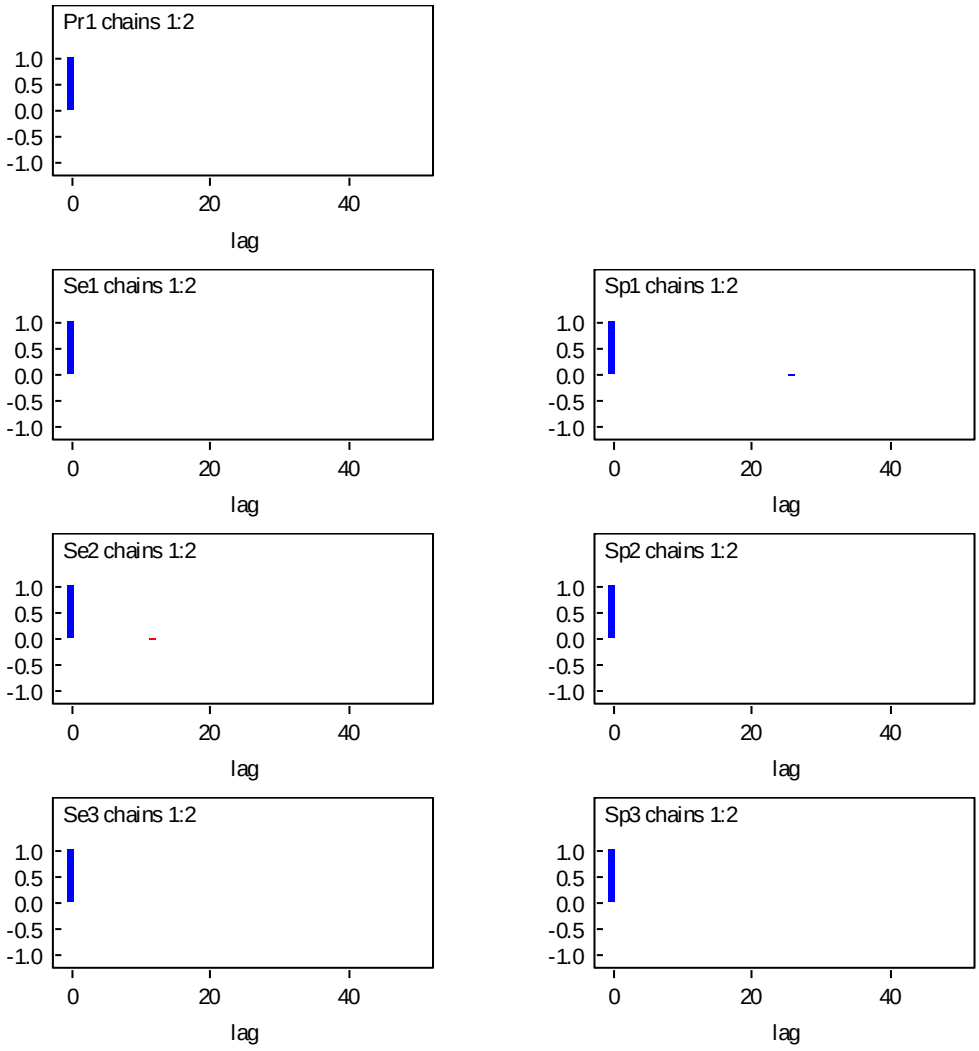
Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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Appendix 10.3.4: Autocorrelation plots for the data set using uninformative priors for prevalence, rRT-PCR, histopathology, and IHC.



Pr1 = Prevalence of disease in the population.

Se1 = Sensitivity of rRT-PCR. Sp1 = Specificity of rRT-PCR.

Se2 = Sensitivity of IHC. Sp2 = Specificity of IHC.

Se3 = Sensitivity of histopathology. Sp3 = Specificity of histopathology.

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APPENDIX 11: Results for Cohen's kappa (unweighted) for histopathology measuring the level of agreement between two observers (reader 1 and reader 2) for the 84 cases where the test results for rRT-PCR, histopathology and IHC were not in perfect agreement (i.e. the results were not all positive or all negative).

In all the tables: Histo = Histopathology.

Appendix 11.1: Cross classified results and Cohen's kappa with respect to the data for all 84 cases.

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	49	10	59
	Positive	13	12	25
	Totals:	62	22	84

Cohen's kappa (Unweighted)	**0.3216**	**(0.1086 - 0.5346)**

Appendix 11.2: Cross classified results and Cohen's kappa with respect to the data for adult ovine and bovine animals.

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	30	2	32
	Positive	7	9	16
	Totals:	37	11	48

Cohen's kappa (Unweighted)	**0.5424**	**(0.2688 - 0.816)**

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Appendix 11.3: Cross classified results and Cohen's kappa with respect to the data for ovine and bovine neonates.

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	13	1	14
	Positive	3	2	5
	Totals:	16	3	19

Cohen's kappa (Unweighted)	**0.377**	**(-0.05024 - 0.8043)**

Appendix 11.4: Cross classified results and Cohen's kappa with respect to the data for ovine and bovine foetuses.

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	6	7	13
	Positive	3	1	4
	Totals:	9	8	17

Cohen's kappa (Unweighted)	**-0.2143**	**(-0.6298 - 0.2012)**

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Appendix 11.5: Cross classified results and Cohen's kappa with respect to the data for mildly autolysed specimens

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	26	6	32
	Positive	5	4	9
	Totals:	31	10	41

Cohen's kappa (Unweighted)	**0.2471**	**(-0.0583 - 0.5525)**

Appendix 11.6: Cross classified results and Cohen's kappa with respect to the data for moderately autolysed specimens

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	8	1	9
	Positive	2	2	4
	Totals:	10	3	13

Cohen's kappa (Unweighted)	**0.4179**	**(-0.1154 - 0.9512)**

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Appendix 11.7: Cross classified results and Cohen's kappa with respect to the data for severely autolysed specimens

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	15	3	18
	Positive	6	6	12
	Totals:	21	9	30

Cohen's kappa (Unweighted)	**0.3478**	**(-0.001461 - 0.6971)**

Appendix 11.8: Cross classified results and Cohen's kappa with respect data for cases where tissue pigments were present

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	137	9	146
	Positive	7	62	69
	Totals:	144	71	215

Cohen's kappa (Unweighted)	0.8306	(0.6969 - 0.9642)

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Appendix 11.9: Cross classified results and Cohen's kappa with respect data for cases where tissue pigments were absent

		Histo results by reader 1		
	Histo results by reader 2	Negative	Positive	
	Negative	100	1	101
	Positive	6	58	64
	Totals:	106	59	165

Cohen's kappa (Unweighted)	0.9094	(0.7571 - 1.062)

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APPENDIX 12: Results for Cohen's kappa (unweighted) for IHC measuring the level of agreement between two observers (reader 1 and reader 2) where the calculations factored in those cases where the test results for rRT-PCR, histopathology and IHC were not in perfect agreement (i.e. the results were not all positive or all negative) as well as all the cases where all the test results obtained by the first reader was in agreement (i.e. all the results for all three tests positive or negative).

In all the tables: IHC = Immunohistochemistry

Appendix 12.1: Cross classified results and Cohen's kappa with respect to the data for all 380 cases.

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	237	10	247
	Positive	13	120	133
	Totals:	250	130	380

Cohen's kappa (Unweighted)	**0.8663**	**(0.7657 - 0.9668)**

Appendix 12.2: Cross classified results and Cohen's kappa with respect to the data for adult ovine and bovine animals.

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	117	2	119
	Positive	7	75	82
	Totals:	124	77	201

Cohen's kappa (Unweighted)	**0.9064**	**(0.7684 - 1.044)**

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Appendix 12.3: Cross classified results and Cohen's kappa with respect to the data for ovine and bovine neonates.

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	58	1	59
	Positive	3	23	26
	Totals:	**61**	**24**	**85**

Cohen's kappa (Unweighted)	**0.8867**	**(0.6745 - 1.099)**

Appendix 12.4: Cross classified results and Cohen's kappa with respect to the data for ovine and bovine foetuses.

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	62	7	69
	Positive	3	22	25
	Totals:	65	29	94

Cohen's kappa (Unweighted)	**0.7408**	**(0.5397 - 0.9418)**

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Appendix 12.5: Cross classified results and Cohen's kappa with respect to the data for mildly autolysed specimens

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	153	6	159
	Positive	5	79	84
	Totals:	158	85	243

Cohen's kappa (Unweighted)	**0.9002**	**(0.7745 - 1.026)**

Appendix 12.6: Cross classified results and Cohen's kappa with respect to the data for moderately autolysed specimens

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	49	1	50
	Positive	2	20	22
	Totals:	51	21	72

Cohen's kappa (Unweighted)	**0.9006**	**(0.6697 - 1.131)**

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Appendix 12.7: Cross classified results and Cohen's kappa with respect to the data for severely autolysed specimens

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	35	3	38
	Positive	6	21	27
	Totals:	41	24	65

Cohen's kappa (Unweighted)	**0.7103**	**(0.4683 - 0.9522)**

Appendix 12.8: Cross classified results and Cohen's kappa with respect data for cases where tissue pigments were present

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	148	1	149
	Positive	2	64	66
	Totals:	150	65	215

Cohen's kappa (Unweighted)	0.9671	(0.8334 - 1.101)

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Appendix 12.9: Cross classified results and Cohen's kappa with respect data for cases where tissue pigments were absent

		IHC results by reader 1		
	IHC results by reader 2	Negative	Positive	
	Negative	104	0	104
	Positive	1	60	61
	Totals:	105	60	165

Cohen's kappa (Unweighted)	0.987	(0.8344 - 1.14)

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APPENDIX 13:

LIEZA ODENDAAL, GEOFFREY T. FOSGATE, MARCO ROMITO, JACOBUS A. W. COETZER AND SARAH J. CLIFT. 2014. Sensitivity and specificity of real-time reverse transcription polymerase chain reaction, histopathology and immunohistochemical labeling for the detection of *Rift Valley fever* virus in naturally infected cattle and sheep. *Journal of Veterinary Diagnostic Investigation.* Volume 26, Issue 1, pp. 49 - 60.

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Journal home page: http://VDI.sagepub.com/

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