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## RESEARCH PAPERS

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# Intra-host mathematical model of chronic wasting disease dynamics in deer (*Odocoileus*)

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**ABSTRACT.** Bioassays of native cervid hosts have established the presence of infectious chronic wasting disease (CWD) prions in saliva, blood, urine, and feces of clinically diseased and pre-clinical infected deer. The intra-host trafficking of prions from the time of initial infection to shedding has been less well defined. We created a discrete-time compartmentalized model to simulate the misfolding catalysis, trafficking, and shedding of infectious prions throughout the organ systems of CWD-infected cervids. Using parameter values derived from experimental infections of North American deer (*Odocoileus spp.*), the exponential-based model predicts prion deposition over time with: 1) nervous tissues containing the highest deposition of prions at 20 months post-infection and 2) excreted fluids containing low levels of prions throughout infection with the highest numbers of prions predicted to be shed in saliva and feces (as high as 10 lethal doses ( $1.34 \times 10^{29}$  prions) in 11–15 months). These findings are comparable to prion deposition described in literature as assayed by conventional and ultrasensitive amplification assays. The comparison of our model to published data suggests that highly sensitive assays (sPMCA, RT-QuIC, and bioassay) are appropriate for early prion detection in bodily fluids and secretions. The model provides a view of intra-host prion catalysis leading to pre-clinical shedding and provides a framework for continued development of antemortem diagnostic methods.

**KEYWORDS.** chronic wasting disease, environmental shedding, horizontal transmission, prion, RT-QuIC, sPMCA, western blot, within-host disease dynamics

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## INTRODUCTION

Chronic wasting disease (CWD) is an invariably fatal, neurodegenerative disease affecting cervids (deer, elk, moose, and reindeer).<sup>1-8</sup> CWD has been confirmed in farmed and free-ranging cervid populations in 24 states, 2 Canadian provinces, South Korea, and Norway,<sup>1,5-7,9-12</sup> and is the only known transmissible spongiform encephalopathy (TSE) to affect both captive and wild cervid populations.<sup>5,9-11,13</sup>

The disease is caused by the accumulation of abnormally folded prion protein (PrP<sup>Res</sup>) that has the capacity to convert additional normal cellular prion precursor protein (PrP<sup>C</sup>) to infectious prion protein (PrP<sup>D</sup>) by a templating mechanism not fully understood.<sup>5-7,14-18</sup> This conversion occurs at the protein level, without underlying genetic changes in the PrP precursor gene.<sup>5</sup> PrP<sup>C</sup> is expressed throughout the body, but is most abundant in nervous tissue.<sup>16</sup> The molecular biomarker for prion diseases (PrP<sup>Res</sup>) can be distinguished from PrP<sup>C</sup> by its partial resistances to proteolysis, tendency to form aggregated plaques, and insolubility in detergents.<sup>6,14,16,19</sup> Other prion diseases include kuru and Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE, mad cow disease) in cattle, and scrapie in sheep and goats.<sup>6,13,16,18,20-21</sup>

In all prion diseases, deposits of PrP<sup>Res</sup> build up in the central nervous system and brain, developing a series of holes histologically resembling a sponge. This structure is termed spongiosis and results in the destruction of neural tissue and function.<sup>7,16</sup> CWD, like all TSEs, has a long preclinical stage of infection during which clinical signs of disease are not apparent (18 months to multiple decades<sup>6,17</sup>), which is followed by a shorter clinical phase of disease (2–6 months) when overt symptoms consistent with TSEs are present.<sup>16,19</sup> Clinical signs of CWD include weight and muscle loss, excessive salivation, and progressively worsening behavioral changes including altered stance, pacing, and hyper-excitability.<sup>2,6</sup> As no therapies or vaccines for CWD exist, death invariably follows.<sup>5,16</sup>

Infectious prions have been detected by bioassay in urine,<sup>11,22</sup> saliva,<sup>8,11-12</sup> feces,<sup>21,23</sup> blood,<sup>12</sup>

antler velvet,<sup>24</sup> and nasal secretions<sup>25</sup> in preclinical and clinical deer and are shed into and contaminate the environment. Even a decaying carcass can act as a reservoir of infectious prions as PrP<sup>Res</sup> has been detected in skeletal and cardiac muscle, reproductive tissues, and fat.<sup>2,6,23,26</sup> CWD can be transmitted through direct contact<sup>5</sup> as well as through airborne routes (aerosolized fecal matter and soil-prion complexes).<sup>3,5,6</sup> In the environment, prions can remain infective for at least 2 years, potentially exposing and infecting a large number of deer indirectly.<sup>5,6,9</sup> As deer consume 8–30 grams (g) of soil per day, depending on the season,<sup>5,23</sup> environmental contamination may play an important role in CWD transmission. The relative importance of these various transmission routes is unclear, but evidence suggests that indirect environmental routes may be the major source for infection.<sup>6</sup> Studies and surveillance indicate that CWD has a low zoonotic potential, but since subclinical CWD-infected deer possess infectious prions, caution is still advisable when handling cervids.<sup>6</sup>

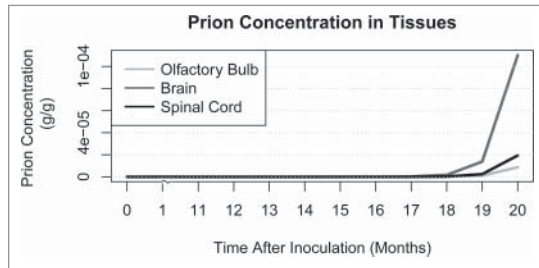
The ecology of CWD at the population level, routes of infection and potential prion shedding mechanism are currently being studied.<sup>3,9,11</sup> What remains less well studied is the intra-host prion trafficking during the protracted incubation period.<sup>11,16,22</sup> The aim of our study was to establish a within-host model describing the patterns of prion trafficking and shedding within mule deer (*Odocoileus hemionus*). With the model, we simulated the timing of environmental shedding of infectious prions through saliva, urine, and feces relative to the onset of clinical symptoms. This understanding allows for the implementation of enhanced antemortem detection methods and management practices to better understand the epidemiology and spread of CWD.

## RESULTS

### *Model prediction of intra-host prion spread*

Using prion transition, replication, and initial invasion parameter values in Supplemental A, we simulated prion spread for 20 months

FIGURE 1. Modeled prion concentration in central nervous system (CNS) tissues: brain, spinal cord, and olfactory bulb. Prion concentrations in all other tissues are at least one order of magnitude lower than that of the CNS.



post-infection to approximate a plausible incubation period for mule deer, which generally exhibit clinical signs 16.2 to 25.9 months after infection.<sup>13</sup> The nervous tissue, namely the brain, spinal cord, and olfactory bulb, contains the highest concentrations of prions at the end of 20 months. All other tissues exhibit prion concentrations at least one order of magnitude lower and are not visible on the same scale (See Fig. 1).

**Model predictions of prion shedding**

The gold standards for detecting prions in tissues are western blot and immunohistochemistry (IHC).<sup>14</sup> We compared the predicted prion concentrations in tissues to the test sensitivity of western blot with a detection threshold of 4 ng/g ( $4 \times 10^{-12}$  g/g).<sup>19</sup> As seen in Table 1, the model predicts that prions accumulate in the brain to this level by 12 months post-infection and should therefore be detectable by western blot. Table 1 also contains model predictions of times to detection via western blot for other tissues commonly

used for CWD-diagnostic tests. While prion infectivity has been found in excreta through bioassay, western blot has been unable to identify PrP<sup>Res</sup> in these samples.<sup>10-12,23</sup> The model predicts that excreted prions in saliva and feces may be detected by western blot by 20 months post-infection while urine is well below the detection threshold at this time (See Fig. 2A).

Recently, more sensitive methods for detecting prions in fluids have been developed.<sup>4,14,18-20</sup> These tests, however, may have higher rates of false positives. Serial protein misfolding cyclic amplification (sPMCA) relies on multiple rounds of incubation of PrP<sup>Res</sup> with excess PrP<sup>C</sup> and sonication to amplify the infectious prions present in a sample.<sup>4,19</sup> With 7 rounds of amplification, sPMCA has the capacity to detect as few as 1.3 ag/g ( $1.3 \times 10^{-18}$  g/g).<sup>19</sup> Real-time quaking-induced conversion (RT-QuIC) relies on a similar method using recombinant PrP<sup>C</sup> substrate and shaking.<sup>20,27</sup> It has a detection threshold of about 1 fg/g ( $1 \times 10^{-15}$  g/g),<sup>20</sup> roughly equivalent to the power of traditional bioassay.<sup>18</sup> Figure 2 illustrates predicted times at which both RT-QuIC (2B) and sPMCA (2C) should detect prions in urine, saliva, and feces.

To further explore the amount of prions shed into the environment during the incubation phase of CWD, we tracked the accumulation of prions in the environment from excreta, including saliva, urine, and feces. As seen in Figure 3, the majority of prions shed into the environment occur through saliva followed closely by prions in feces. By 11 months post-infection, the number of prions shed into the environment through excreta constitutes one lethal intracerebral experimental dose ( $1 \text{ fg}^{18}$  or  $1.34 \times 10^{28}$  prions assuming the molecular weight of PrP<sup>Res</sup> is 27 kDa<sup>25</sup>). Within another month, the total number of prions shed into the

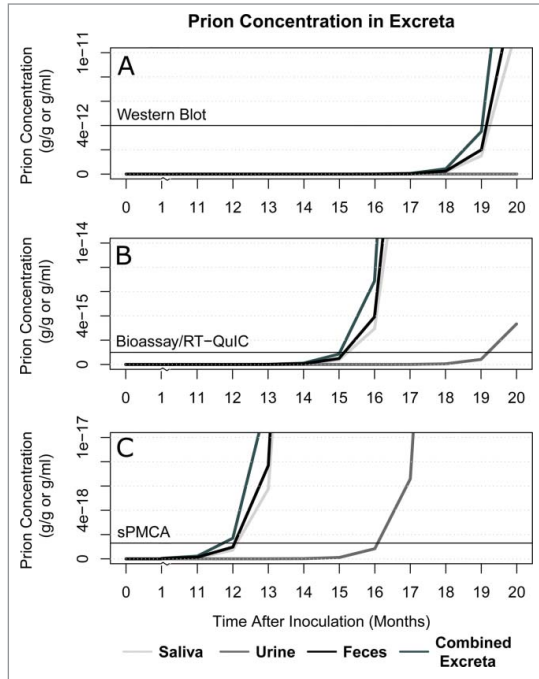
TABLE 1. Model predicts mean time (mpi)<sup>†</sup> to CWD detection by western blot in various tissues.

RPLN	Saliva	Tonsils	Peyer's Patches	Brain	Urine	Rectal Follicles	Feces
20	20	19	17	12	NA <sup>‡</sup>	18	20

<sup>\*</sup>Months post-inoculation

<sup>‡</sup>NA, does not occur before 20 months post-inoculation

FIGURE 2. Predicted detection of prions in excreta (urine, saliva, feces, and a combined pool of all 3). The three panels show detection thresholds of (A) western blot (4 ng/g), (B) bioassay and RT-QuIC (1 fg/g), and (C) sPMCA (1.3 ag/g) against the predicted prion concentrations in excreta. (Color figure available online.)

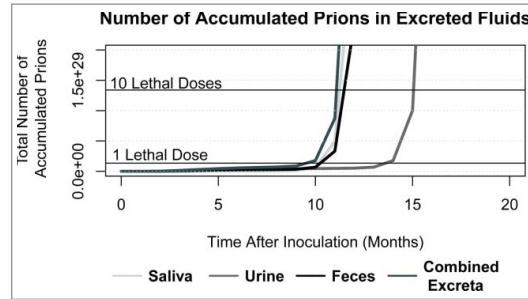


environment by excreta since inoculation reaches 10 lethal doses ( $1.34 \times 10^{29}$  prions).

### Sensitivity of model parameters

We tested sensitivity of response variables to parameter values used in the model. For the main response of interest—time (months) after infection until 10 lethal doses ( $1.34 \times 10^{29}$  prions) accumulated in the environment via excreted fluids—10 of the 78 model parameters changed the outcome of the model (see Fig. 4). Sensitive transition parameters included the transition of prions to and from the brain and from the olfactory bulb to swallowed and orally excreted saliva. Sensitive replication parameters included replication of prions in the brain and rectal follicles. During initial uptake of prions, invasion of the lymph and blood were the most sensitive.

FIGURE 3. Predicted number of total accumulated prions in excreta (saliva, urine, feces, and a combined pool of all 3). Horizontal lines indicate the equivalent number of prions in one and 10 experimental intracerebral lethal doses ( $1.34 \times 10^{28,29}$  prions). (Color figure available online.)



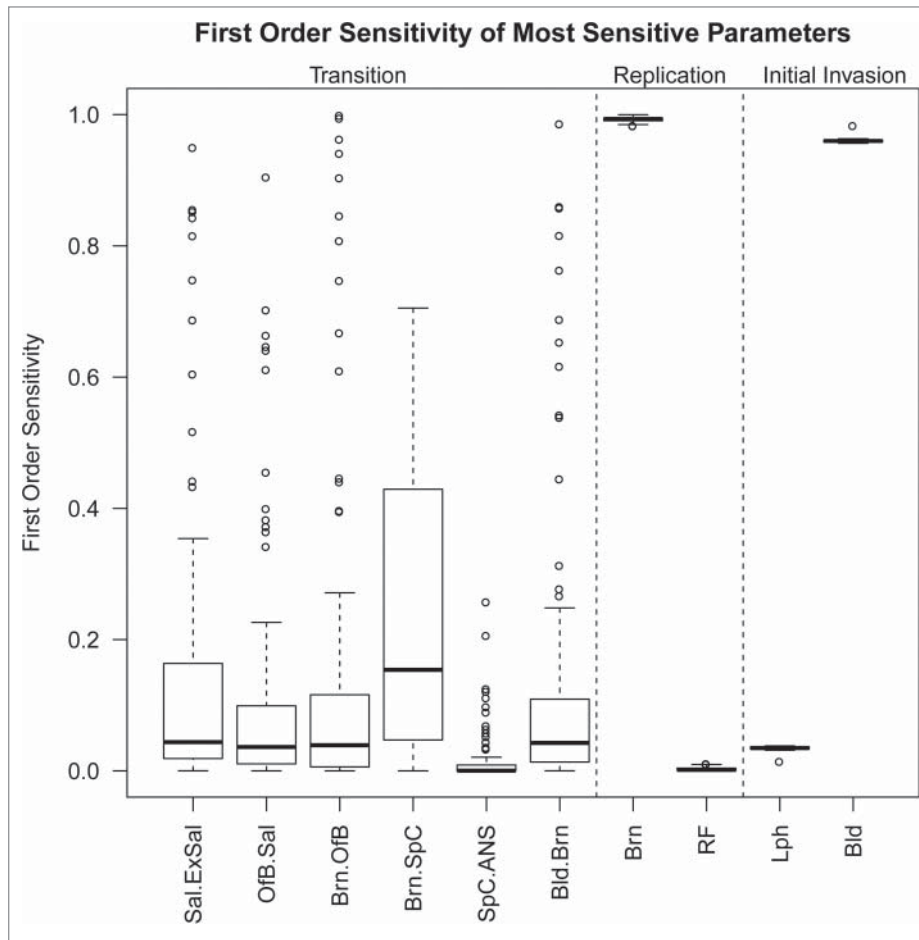
Using the range of parameter values shown in Supplemental A, the range of post-infection times required to reach environmental accumulation of 10 lethal doses ( $1.34 \times 10^{29}$  prions) ranged from 10–18 months (data not shown).

## DISCUSSION

Our aim was to develop a model describing intra-host prion trafficking and shedding of prions, an area lacking in CWD literature. Using literature-derived values and assumptions pertaining to the temporal and spatial distribution of PrP<sup>C</sup> and PrP<sup>Res</sup>, the organ systems implicated in disease progression and detection of prions in excreta, the model describes the complete system and pattern of intra-host trafficking from infection to shedding. The model predicts that the brain, olfactory bulb, and spinal cord contain the highest deposition of prions by the end of a 20 month incubation period and that throughout this period, prions are shed through saliva, urine, and feces. During the majority of the incubation period, the model predicts that prions cannot be detected via western blot in the excreta. Predicted prion concentrations in saliva, however, are detectable by sPMCA and RT-QuIC as early as 11 and 15 months post-infection, respectively.

The sensitivity analysis helps to validate our model. The sensitivity of the model to replication in the brain suggests that this is a location where a

FIGURE 4. First order sensitivity of the 10 most sensitive parameters in the model. Parameters that have a range that do not contain zero and whose maximum sensitivity are greater than  $1 \times 10^{-3}$  are considered the most sensitive parameters. Parameters are grouped by the ‘class’ of model parameters to which they belong: transition, replication or initial invasion. Transition parameters are labeled  $x,y$ , indicating movement of prions from tissue  $x$  to tissue  $y$ . Outliers, defined as data points outside of 1.5 interquartile ranges of the first and third quartiles, are indicated by points outside of the whiskers. Sal, saliva; ExSal, excreted saliva; OfB, olfactory bulb; Brn, brain; SpC, spinal cord; ANS, autonomic nervous system; Bld, blood; RF, rectal follicles; Lph, lymph.



large amount of PrP<sup>C</sup> to PrP<sup>D</sup> conversion occurs *in vivo*. This corresponds to known high concentrations of conversion-ready PrP<sup>C</sup> in the central nervous system (CNS).<sup>16</sup> The high model sensitivity to replication in the brain indicates that levels of shed prions depends strongly upon prion replication in the brain, even though these CNS prions must then pass through other organ systems to be shed. Physiologically, replication in the brain results in the development of plaques and spongiosis of neural tissue, resulting in clinical disease.

This model suggests that large accumulation of shed prions occurs late in infection, likely localizing the environmental reservoir and facilitating CWD horizontal transmission. This corresponds to observed spatial clustering of CWD cases.<sup>6,28</sup> Transition parameters with high sensitivity included the movement of prions from the brain to excreted saliva, and the movement of prions through the CNS. This implicates salivary shedding as the most important source of environmental prions. It also predicts that the majority of

infectious prions originate in the CNS. It has been shown that feces and saliva contain similar infectivity, and that infectivity is greater than that of urine.<sup>8,14</sup> Similarly, the rate of initial prion invasion into the blood and lymph also influences the environmental pool of prions, and illustrates that the earlier prions enter the circulatory system, the earlier shedding will occur; potentially because the blood widely disseminates prions throughout bodily systems. Prions can be detected in the blood within minutes of inoculation and remain throughout the infection,<sup>27</sup> illustrating the importance of blood in prion dissemination.

Corroborating earlier studies,<sup>7,11</sup> this model assumes exponential deposition of prions in tissues over time and predicts that the brain, olfactory bulb, and spinal cord contain the highest accumulation at the end of 20 months. As previously illustrated in experimental CWD studies, western blot detects prions in the obex of the brainstem by 16–22 months post-infection<sup>29</sup> although another study did not detect prions 19 months post-infection.<sup>10</sup> As seen in Table 1, our model predicts the brain will test CWD-positive by western blot by 12 months post-infection. This suggests that the predicted rate of prion accumulation is faster than experimentally reported. Further, the model predicts that western blot will test CWD-positive in tonsils by 19 months post-infection and in rectal mucosa lymphoid tissue by 18 months post-infection (see Table 1). Western blot CWD-positivity has been reported in tonsils 6 months post-infection and 6–12 months post-infection in rectal mucosa lymphoid tissue.<sup>3</sup> It appears that the model either over-estimates prion spread into and replication within the neural tissues, or under-estimates prion dissemination from these tissues. Alternatively, but not exclusively, the model may under-estimate prion transition to and replication within lymphatic compartments or over-estimate the loss of prions from these compartments. This highlights the need for further experimental observation of these rates.

The predicted prion concentration within the nervous system is biologically impossible. As seen in Figure 1, the final PrP<sup>Res</sup> concentration in the brain is about 0.1 mg/g while the spinal cord and olfactory bulb have PrP<sup>Res</sup> concentrations between 10  $\mu$ g/g and 20  $\mu$ g/g. This is not biologically possible and thus, the model

prediction may result from a lack of evidence describing these *in vivo* transition parameters. Moreover, an as-of-yet unknown and unincorporated variable or mechanism may prevent the model from producing completely realistic predictions. Additionally, replication parameters remain the same over the course of the simulation. It has been shown that as the disease progresses, the pool of available cellular prions decrease as PrP<sup>D</sup> converts PrP<sup>C</sup>,<sup>17</sup> thus reducing prion replication rate within organs. In nature, we would hypothesize that both the transition and replication parameters for tissues would vary over the course of infection.

While the manner in which prions transition between compartments is unknown, we assumed the most general case, namely that prions traffic between tissue compartments. However, since the *in vivo* parameter values are unknown, it was difficult to determine if this scenario of prion movement and misfolding catalysis reflects the actual prion transitions *in vivo*. Further research is needed to clarify this step.

As illustrated in Figure 2A, western blot analysis does not have the required sensitivity to detect prions in fluids until late in CWD incubation and is thus not an effective diagnostic tool. RT-QuIC, bioassay, and sPMCA have detection thresholds 3 to 6 orders of magnitude lower than western blot<sup>19</sup> (see Figure 2B and C). Thus, these techniques are better tools for early detection of TSEs.<sup>13,18–20,29</sup> Bioassay has detected prions in saliva at 6–18 months post-infection,<sup>8,12</sup> and in feces at 9 months post-infection.<sup>23</sup> Prions have been detected in urine by RT-QuIC as early as 13–16 months post-infection.<sup>13</sup> Our model predicts that saliva and feces should test positive by bioassay and RT-QuIC at 15 months while urine is positive at 19 months (see Fig. 2B). Thus, our model predicts lower prion concentrations in urine and feces than experimentally observed while predicting saliva concentrations at the lower end of observed concentrations.

Given that the model predicts lower prion concentrations in excreted fluids than observed, Figure 3 represents a conservative view of pre-symptomatic shedding. We predict that 10–14 months is required for one lethal dose of prions ( $1.34 \times 10^{28}$  prions) to be shed via

saliva, feces, or urine. On the other hand, a 10-fold increase in number of prions in excreta occurs within one to 2 months after the accumulation of one lethal dose. It is easy to see that the number of excreted prions could equal those found in the terminal brain very quickly.<sup>8</sup> The model predicts a large amount of shedding in the later part of infection with the bulk of shed prions deriving from saliva and feces. This highlights a potential route of direct transmission through social encounters and grooming where infectious saliva, urine or feces may transfer between individuals. Because females tend to congregate in herds more frequently than males,<sup>30</sup> direct contact with saliva and feces shed by CWD-positive individuals may have a larger impact on transmission dynamics in females than in males. However, males may inhale more environmental CWD particles because they engage in more behaviors that generate dust.<sup>5</sup> Additionally, the flehmen response in males may efficiently expose them to large quantities of infectious urine, saliva and feces during the rut.<sup>14</sup> Indirect environmental contamination should be a route of transmission for both sexes.

Intra-host prion trafficking rates and the manner in which these transitions occur needs further exploration. However, this model's novel description of intra-host trafficking reinforces the importance of pre-clinical shedding and implicates saliva and feces as the primary sources of prions for transmission, which occurs mainly in later stages of CWD incubation. This means that direct contact with excreta through grooming and social interactions may be important mechanism of transmission. Even with the model's conservative prediction of shedding through saliva, urine, and feces, the number of prions excreted pre-clinically by a single infected animal can reach 10 lethal doses, quickly producing a large environmental prion pool. The model also indicates that while western blot is useful in validating the presence of prions once clinical disease is obvious, a more sensitive test is needed to identify early prion infection. RT-QuIC, sPMCA, and bioassay are more appropriate in detecting low levels of prions in excreta in the early stages of CWD. Overall, the model provides

insight into intra-host CWD disease dynamics and may provide assistance in the development of antemortem detection methodologies and strategies for the management of CWD in cervid populations.

## MATERIALS AND METHODS

### *Biological structure of the model*

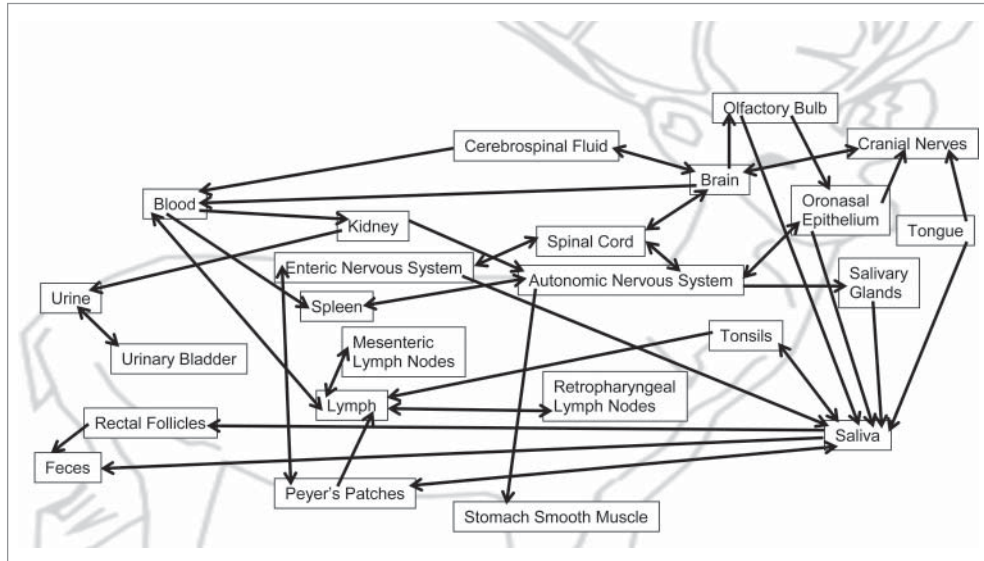
The model parameters mirror an infected female mule deer in terms of organ size and transition parameters gleaned from published experimental infections. Where information on mule deer was not available, data from white-tailed deer were substituted. Mule deer and white-tailed deer are phylogenetic sister species,<sup>31-32</sup> are very similar physically,<sup>31</sup> and thus acquire CWD similarly. The model was coded and analyzed using version 3.2.2 of the R programming package (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>).

The current understanding of intra-host CWD trafficking suggests that PrP<sup>D</sup> spreads from the peripheral infection site (generally lymphoid tissues) to the central nervous system via neural and lymphatic pathways where amplification occurs.<sup>15,33,34</sup> Infectious prions then spread back to peripheral tissues.<sup>10-11,25</sup> After initial infection, prion deposits grow throughout the cervid bodily systems as PrP<sup>C</sup> is misfolded into PrP<sup>D</sup>. We limit possible routes of prion transmission to physically interacting or adjacent organs as outlined in Figure 5. Our work aims to clarify the relative importance of these many potential pathways.

Our model assumes oral inoculation, as is most common for *in vivo* laboratory CWD investigations. Ingested material passes through the oronasal cavities and then progresses through the gastrointestinal (GI) tract. In order to gain entry to the body, prions must cross the mucosal epithelium into bodily fluids or become deposited in the oronasal epithelium. Prions cross the epithelium and are moved to lymph by paracellular transport.<sup>2,15</sup> Prions also pass into blood within minutes of oral exposure<sup>27</sup> and can be taken up by lymphoid tissue (tonsils and Peyer's patches) that continually



FIGURE 5. Current hypothesized spread of PrP<sup>D</sup> in mule deer. Prions can transition between organs and fluids as CWD progresses. A single-headed arrow indicates that prions only transition in that direction while a double-headed arrow indicates that prion transition occurs in both directions between those tissues.



sample the GI environment for foreign material.<sup>5,16,21,33</sup> Both routes deposit PrP<sup>D</sup> into the lymph, passing through regional lymph nodes before draining back into the circulatory system. In circulation, prion-infected blood passes through the spleen, depositing prions.<sup>2</sup> Prions enter the nervous system from the sympathetically innervated spleen<sup>7</sup> or Peyer's patch-associated enteric nerves.<sup>16</sup> Autonomic and enteric nerves are thought to allow PrP<sup>D</sup> to spread to and amplify within the spinal cord before invading the brain.<sup>2,7</sup> Neuroinvasion can also occur directly through cranial nerves in the tongue and oronasal epithelium, but this process appears to be slower than the lymphatic route.<sup>8,33</sup> After amplification in the brain, prions spread back through the autonomic and enteric nervous systems to infect peripheral tissues like skeletal muscle, kidneys, and salivary glands.<sup>2</sup> Within the olfactory bulb of the brain, prions pass through olfactory receptor neurons, which amplify PrP<sup>D</sup> to the oronasal epithelium.<sup>5,25</sup> The brain can also receive prions from the blood at the circumventricular organs which lack the blood-brain barrier.<sup>4</sup> As the disease progresses, tissues throughout the brain deteriorate and prions can leak through the ependyma into the cerebrospinal fluid.

Shedding prions into saliva occurs from tissues associated with the GI tract, like the tongue, salivary glands, lymphoid tissues, and enteric and olfactory neurons.<sup>21,25,34</sup> Deer swallow the majority of their saliva, leading to potential increased prion deposition into tissues of the GI tract.<sup>2,8</sup> The presence of prions in urine may arise from the filtrate passing through the kidneys as well as infectious prions shed from the kidneys and urinary bladder. Urine titers of PrP<sup>Res</sup> are very similar to those of the blood, indicating that the majority of PrP<sup>Res</sup> present derives from the blood.<sup>22</sup>

In order for prion deposition to occur across bodily organs and fluids, prions must transition between organs and fluids that interact with each other; prions cannot directly "jump" to non-adjacent tissues. In the instances when prion accumulation occurs in physically disparate tissues, bodily fluids like blood or lymph must be responsible for the prion transport. Prion transition between organs may occur by 2 methods: by directly moving into the adjacent organ or by catalyzing the conversion of PrP<sup>C</sup> to PrP<sup>D</sup> at the cellular border between organs without the prion catalyst actually exiting the original host tissue. It is unclear which method occurs in natural



CWD infection, which is reflected in the use of ambiguous terms such as “prion spread”<sup>7</sup> and “physical interactions.”<sup>16</sup> Other experiments support the idea that PrP<sup>D</sup> must come in direct contact with PrP<sup>C</sup> in order to convert it,<sup>15</sup> thus implying that prions must physically move between tissues. To provide the most general model of prion transitions, our model assumes that prions always physically move between tissues, or undergo “border crossing,” for all transitions. Other possible transition schemes might include that prions only “border cross” in and out of fluids (shedding into and deposition by fluids) or only “border cross” into fluids (shedding into fluids) while at all other interfaces, prions simply catalyze conversion of PrP<sup>C</sup> to PrP<sup>D</sup> in adjacent tissues without leaving the host tissue. All three possible transition schemes are included in the R code in Supplemental C, but in this report we present and discuss only the most general transition scheme where prions always “border cross” between all tissues.

The amount of prion deposition in each organ depends on the pre-existing amount of prion precursor available in the organ to be converted. The brain and nervous tissue contain the highest

each of 27 tissue compartments. We assume that prion transitions between compartments occur before prion replication. Assuming the oral inoculation route, initial prion distribution at  $t=0$  is determined by the portion of infectious inoculum taken up by tissues along the GI tract (see Supplemental A for values). To correspond with experimental infections, all modeled infections begin with one lethal intracerebral dose of PrP<sup>D</sup> (1 fg<sup>18</sup> or  $1.34 \times 10^{28}$  PrP<sup>D</sup> proteins, assuming the molecular weight of PrP<sup>Res</sup> is 27 kDa<sup>25</sup>).<sup>22</sup> We assumed that initial tissue uptake of prions is temporally instantaneous relative to the model’s monthly time scale; no unassimilated inoculum remains after the first time step.

Transition parameters, which illustrate the “border crossing” of prions between tissue compartments, are stored in matrix **A** (see Supplemental B). For example, see below a row of the transition matrix **A** ( $A_{ANS}$ ) which corresponds to prion transitions out of the autonomic nervous system (ANS). Tissues into which prions transition from the ANS are listed above the associated transition parameters.

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$A_{ANS} =$	<i>Epi</i>	...	<i>Lph</i>	<i>SGd</i>	...	<i>SpC</i>	<i>CSF</i>	<i>Bld</i>	<i>Kd</i>	...
	0.03	...	0	$1 \times 10^{-3}$	...	0.2	0	0	$1 \times 10^{-3}$	...

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concentrations of cellular prion precursor<sup>16</sup> so these would have the largest deposition of prions. All other tissues have at least 10x lower concentration of cellular prion precursor than the brain while fluids contain none.

**Mathematical structure of model**

We modeled the spread of prions during infection as a discrete time, compartmentalized model. The growth within and transition between compartments at each monthly time step ( $t$ ) is given by:

$$p_{t+1} = p_t * A \tag{1}$$

At each time step, vector  $p_t$ , where  $p_t$  is of length 27, represents the number of prions in

Transition parameters for organ-to-organ and fluid-to-organ interactions take into account the proximity of tissues, relative size of organs, and level of PrP<sup>C</sup>. Tissues that are not physically in contact, like the ANS and cerebrospinal fluid (CSF), have no direct “border crossing” between them so the corresponding transition parameter between the ANS and CSF ( $\beta_{ANS.CSF}$ ) equals zero. Also, the relative size and amount of surface area in contact between adjacent tissue compartments inform the degree to which prion “border crossings” are possible. As publications do not report surface areas or overlaps between compartments, estimates were based on relative organ masses as well as physiological co-location and function of organs that would facilitate overlapping. For example, the oronasal epithelium, a

large tissue compartment, would share a larger total amount of surface area with the ANS than the salivary glands, a small compartment, because of the larger number of innervations the ANS has with the oronasal epithelium (See Table 2 for organ masses). Thus, the transition parameter for prions “border crossing” from the ANS into the oronasal epithelium ( $\beta_{ANS.Epi} = 0.03$ ) is larger than that of the ANS into the salivary glands ( $\beta_{ANS.SGd} = 1 \times 10^{-3}$ ).

Transition parameters for organ-to-fluid interactions encompass cellular turnover rates, as well as prion concentrations in the organs. During normal

cellular turnover, apoptosis is thought to release PrP<sup>D</sup> and PrP<sup>C</sup> into the surrounding bodily fluids of blood, lymph, saliva, urine, and feces.<sup>9</sup> Since nervous tissue contains at least 10x more prion precursor than other tissues, transition parameters for nervous tissue to fluid “border crossing” is larger than that of prions entering fluids from non-nervous tissue. For example, the mean value for transition from the olfactory bulb neurons to saliva is 0.5 while the mean value for Peyer’s patches to saliva is 0.05 (see Supplemental B).

Growth terms along the diagonal of **A** incorporate both the growth within and the movement

TABLE 2. Organ mass and volume of fluids present monthly in mule deer.

Organ or Fluid	Mass of organ (g)	Volume of Fluid (ml)	Reference and Assumptions
Oronasal Epithelium	1,000	—	(35)
Tongue	63	—	(36)
Retropharyngeal Lymph Nodes	15	—	Assumed to be smaller than tonsils
Spleen	200	—	(37)
Lymph	—	2,000	Assumed volume of lymph to be equal to ½ blood volume
Salivary Glands	20	—	Assumed size and number similar to tonsils
Saliva	—	40,5000	(8)
Tonsils	20	—	Assume to be size of ½ hockey puck
Peyer’s Patches	20	—	Assumed to be 5% of ileum mass which is 25% of total GI mass (36)
Stomach Smooth Muscle	100	—	Estimated based on total 30 kg mass of skeletal muscle (36)
Olfactory Bulb	60	—	<a href="http://www.imbmonsterbucks.com/contents/info.php?id=243">http://www.imbmonsterbucks.com/contents/info.php?id=243</a>
Autonomic Nervous System	100	—	Assume 100 million neurons in system with average mass of $10^{-6}$ g (38)
Enteric Nervous System	100	—	Assume 100 million neurons in system with average mass of $10^{-6}$ g (38)
Cranial Nerves	1	—	Assume 12 nerve pair network approx. 1 g (36)
Brain	350	—	
Spinal Cord	50	—	Assumed to be small compared to brain, approx. 1/7 mass of brain
Cerebrospinal Fluid	—	330	Based on 1:0.02 human brain: cerebrospinal fluid ratio (36)
Blood	—	4,000	Assuming 4L of blood present (36)
Kidneys	443	—	
Urinary Bladder	10	—	Assumed to be small organ, but thick walled for expansion (5)
Urine	—	8,333	
Rectal Follicles	10	—	Assumed to be smaller and less numerous than tonsils (23)
Feces	23,4000	—	

between compartments of prions. The amount of PrP<sup>C</sup> present dictates the degree to which prions can catalyze prion precursor misfolding and thus replicate in that tissue. A high level of prion precursor provides a large substrate pool for prion catalysis, resulting in large increases in prion number and a comparatively larger replication term than a compartment with a lower level of prion precursor. For example, the urinary bladder, which has 10-times less prion precursor than the brain, has a replication term of 2.0 while the kidneys, which have 20 to 100-times less prion precursor than the brain, have a replication term of 1.1. See Supplemental A for the complete list of parameter values. As fluids do not contain endogenous PrP<sup>C</sup> other than that shed from surrounding tissues, the replication term for fluids is 1; prions can only enter these tissues by “border crossing,” not by within-tissue conversion of PrP<sup>C</sup>. Because our model assumes that prions physically leave tissues during the transition phase, there are fewer prions left in the compartment during the replication phase and the growth term in the diagonal of **A** is only a portion of the replication term for that compartment. For example, if compartment *x* has replication term,  $r_x$  but 20% of the prions leave compartment *x* each month, the diagonal of **A** for compartment *x* ( $A_{x,x}$ ) is:  $r_x * (1 - 0.2)$ .

While the model uses prion counts for the calculation of spread of prions throughout tissues, we also calculate concentrations within tissue compartments to allow for comparison to published values, specifically when determining predicted time to detection by methods of known detection thresholds. For this conversion, prion molecular weight was assumed to be 27 kDa.<sup>25</sup> Table 2 contains a list of the mass or volume of each organ and fluid. For fluids that are continually excreted, the total volume produced per month is used.

Parameter values used for calculating the spread of prions throughout the mule deer are listed in Table 2 (mean parameter values).

**Sensitivity analysis**

We evaluated first order sensitivity of parameters using ‘Distributed Evaluation of

Local Sensitivity Analysis’ (function ‘delsa’) in the sensitivity package (<https://cran.r-project.org/web/packages/sensitivity/sensitivity.pdf>) of the program R. The function scaled sensitivity for each parameter by the variance in the response variable attributed to that parameter divided by the total variance in the response variable for all inputted parameters. We declared the response variable to be the number of months post-infection required to accumulate 10 lethal doses ( $1.34 \times 10^{29}$  prions) of shed prions in the environment. Parameters belonged in 3 ‘classes’—transition, replication, and initial invasion—in order to simplify analysis and provide validation for each part of the model. We analyzed sensitivity on each parameter class individually. See parameter means and ranges used for sensitivity analysis in Supplemental A.

**ABBREVIATIONS**

ANS	autonomic nervous system
Bld	blood
Brn	brain
CN	cranial nerves
CNS	central nervous system
CSF	cerebrospinal fluid
CWD	chronic wasting disease
ENS	enteric nervous system
Epi	oronasal epithelium
ExFcs	excreted feces
ExSal	excreted saliva
ExUrn	excreted urine
Fcs	feces
GI	gastrointestinal
IHC	immunohistochemistry
K <sub>d</sub>	kidney
Lph	lymph
MLN	mesenteric lymph nodes
OfB	olfactory bulb
PP	Peyer’s patches
PrP <sup>C</sup>	cellular prion protein
PrP <sup>D</sup>	disease forming aberrant prion
PrP <sup>Res</sup>	protein kinase resistant prion protein of unknown infectivity detected by assays
RF	rectal follicles
RPLN	retropharyngeal lymph nodes

RT-QuIC	real-time quaking-induced conversion
Sal	saliva
SGd	salivary glands
SpC	spinal cord
Spl	spleen
sPMCA	serial protein misfolding cyclic amplification
SSM	stomach smooth muscle
Tng	tongue
TSE	transmissible spongiform encephalopathy
Tsl	tonsils
UB	urinary bladder
Urn	urine

### **DISCLOSURE OF POTENTIAL OF CONFLICTS OF INTEREST**

No potential of conflicts of interest were disclosed.

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