On the role of feral ruminants in the transmission of bovine herpesvirus 1 to domestic cattle

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Elisabeth Mollema

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Abstract

There is an ongoing debate in The Netherlands between farmer organisations, conservationists and government about whether the health status of feral animals jeopardises the health status of domestic cattle. In this respect, BHV1 is the most prominent acute problem. Although the compulsory eradication programme for BHV1 in domestic cattle populations is suspended, eradication of BHV1 still takes place on a voluntary basis.

The objective of this thesis was to study the role of Heck cattle and red deer living in Dutch nature reserves for the possible introduction of BHV1 in domestic cattle. Therefore, the research question whether BHV1 could persist in Heck cattle and red deer populations had to be answered.

The persistence of BHV1 in small cattle populations was studied by estimating the mean time to extinction using a stochastic model. For realistic parameter values, it was found that the mean time to extinction was already in the order of 100 years in a population of 10 animals. As the contact structure of the Heck cattle populations may influence BHV1 transmission and thus also BHV1 persistence, this contact structure was quantified using behaviour observations. It was shown that the contact structure was more limited for transmission of BHV1 in the autumn and winter-spring period than in the summer period. During summer most transmission would take place based on the number of contacts. The effect of vaccination was then studied on the dynamics and persistence of BHV1 in the Heck cattle populations. Serological data of BHV1 in the Heck cattle populations were combined with model simulations. From the serological data and simulations results it was clear that vaccination of a large part of the Heck cattle population decreased the seroprevalence for BHV1. It was also found that in 3 out of 20 simulated populations, BHV1 became already extinct within 15 years for the partly vaccinated populations. For the red deer populations it was quantified to what extent BHV1 might spread among red deer using two transmission experiments. It was shown that red deer can be infected with BHV1 and excrete BHV1, but no transmission of BHV1 was observed among red deer.

From the results it could be concluded that BHV1 will persist in the Heck cattle populations if no eradication measures were taken but will not persist in red deer populations. However, this does not necessarily imply that, for the eradication of BHV1 in domestic cattle, eradication of BHV1 in the Heck cattle populations is necessary.

Contents


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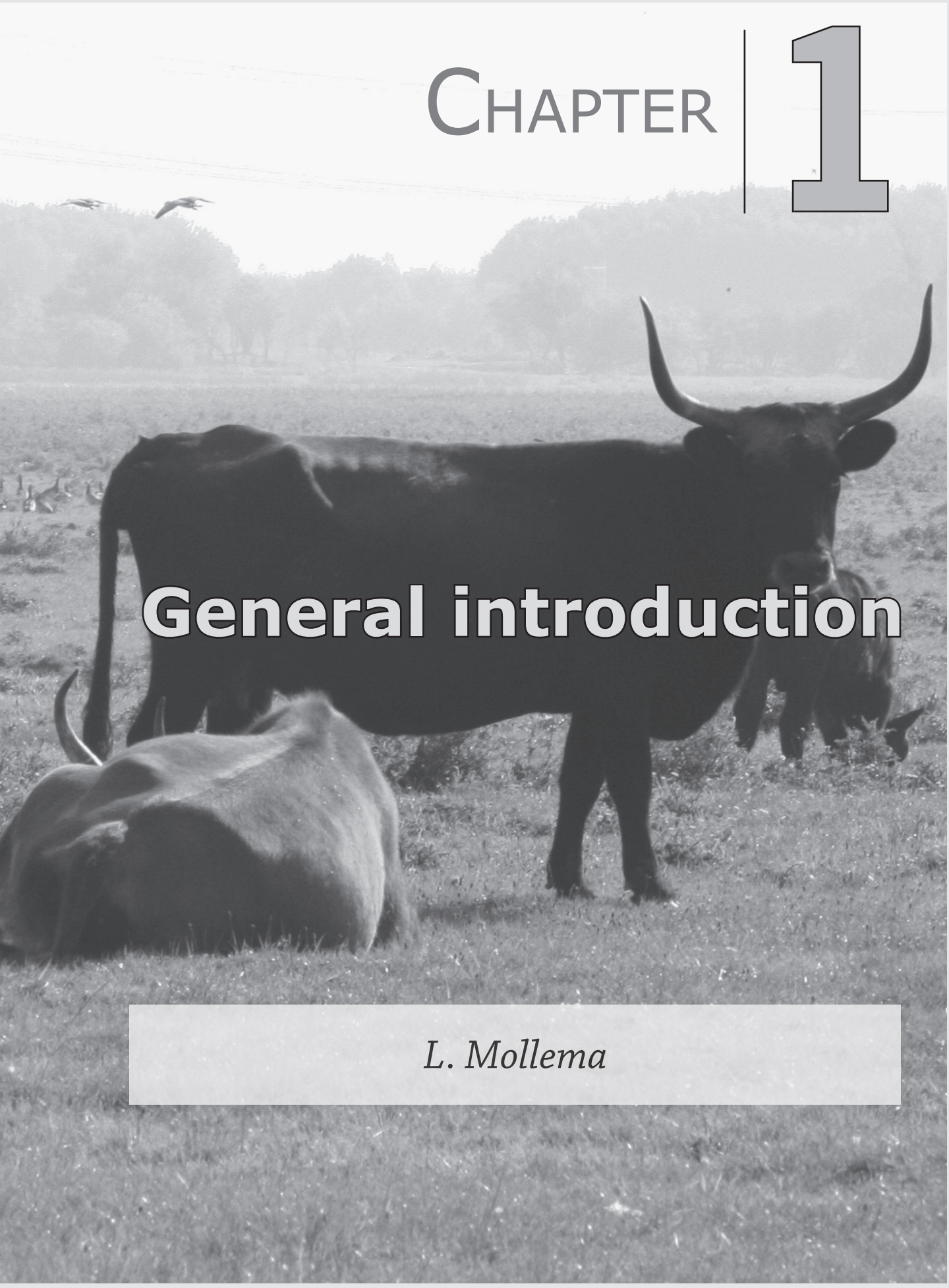

Chapter 1

General introduction

L. Mollema

Setting the stage

Various reasons do exist to study infectious diseases among wildlife. To be mentioned are the following.

i) the diseases are zoonoses that are transmitted directly from wildlife to humans (e.g. rabies and lyme disease).

ii) wildlife can be a reservoir for pathogens causing these diseases and can therefore prevent the eradication of these pathogens among domestic animals (e.g. bovine tuberculosis and brucellosis).

iii) there may exist general concern about the conservation of wildlife populations that are, besides by diseases, also affected by habitat degradation, fragmentation, and habitat loss (Wobeser, 2000 and references therein).

Our focus will be on infectious diseases that have to be eradicated among domestic animals because of zoonotic aspects of the disease, and/or economical loss as a consequence of morbidity and mortality among the animals or as a consequence of a ban on the export of animal products. For an infectious disease that has to be eradicated among domestic animals, the question arises whether wildlife will make the eradication more difficult or even impossible. Wildlife can only hamper the eradication when firstly the infectious pathogen persists in the wildlife population and secondly transmission occurs between wildlife and domestic animal populations.

This thesis focuses on possible persistence and eradication of an infectious pathogen in wildlife. A necessary but not sufficient condition for the survival of an infectious pathogen in a population is that the reproduction ratio (R) is above one. The reproduction ratio is the average number of newly infected animals caused by one typical infected animal (Diekmann et al., 1990). For an infectious pathogen to persist in a population there needs to be a sufficient influx of susceptible animals in the population, next to the condition of $R > 1$. Below, various aspects will be addressed of studying the role of wildlife species in the transmission of an infectious pathogen that needs to be eradicated in domestic animals.

Susceptible wildlife species

The first step is to identify wildlife species, which can be infected with a particular pathogen. Candidate species are first of all those that are taxonomically related to the domestic species but also those that have direct or indirect contact with the domestic species under study. For example badgers and cattle may use the same resources provided by cattle farms. It is

suggested that infectious badgers may transmit bovine tuberculosis to cattle by making direct respiratory contact or indirect through contamination of the premises (e.g. feed sheds, cattle troughs) with badger excreta and secretions (Garnett et al., 2002). The next step is then to look for the presence of the pathogen in the identified susceptible wildlife species. Ante-mortem and post-mortem diagnosis (clinical signs, cell-mediated immune-based tests, serological tests, isolation of the pathogen, rapid DNA amplification procedures (PCR), DNA fingerprinting) can be used to identify the (past) presence of the pathogen in the wildlife species. However, it is questionable whether techniques used for detection of infectious pathogens in humans or domestic animals can also be used to prove the existence of the same pathogens in wildlife species. Difficulties may arise such as that the development of disease in wildlife differs from that in the domestic species or that tests used, cross-react with pathogens naturally found in the wildlife species. Therefore, tests that have been developed for domestic animal populations should be very well validated in wildlife populations before they can be used at large scale in wildlife.

Core and satellite groups

If the infectious pathogen has been traced in wildlife species, then the next step in studying the role of that species in the transmission of a pathogen is to distinguish between core and satellite groups. A core group is defined as a group of any smallest combination of populations for which R is larger than one. Any group not belonging to a core group, but which can become infected, is called a satellite group. For satellite groups, R is less than one. If $R < 1$, then each case does not replace itself on average and the disease will die out. If $R > 1$, then the disease may spread.

Given the distinction between core and satellite groups it is to be investigated whether the disease can spread within the wildlife species and to what extent. For that purpose the reproduction ratio (R) has to be estimated. Transmission experiments have been used to estimate R of an infectious pathogen in domestic animals (Greenwood et al., 1936; Kermack and Mckendrick, 1936; De Jong and Kimman, 1994; Bouma, 1997). These experiments are less applicable to (large) wildlife species because of technical problems with, for example, handling and housing of animals and difficulties in fulfilling the ethical directives for animal experiments. Other data on host-pathogen dynamics such as serological data, case notification reports, mortality data, observational data or outbreak data from cross sectional surveys or longitudinal (cohort) studies can be used to estimate R. However, it is also not very easy to collect these data from wildlife populations. For example, the collection of blood samples in feral animals may cause considerable stress. Another problem that may arise,

is the estimation of the number of feral animals that have been exposed to the pathogen, which is necessary for determining the population at risk. For the analysis of transmission data, stochastic (Markov models or chain-binomial models) and deterministic (ordinary differential equations or partial differential equations) epidemic models can be used together with statistical methods such as least mean squares, maximum likelihood, zero-mean martingales or a Bayesian approach. See also Becker (1989) for applications of these statistical methods to infectious disease data.

Persistence

If a pathogen is able to spread within a wildlife population ($R > 1$) it still should be investigated whether the pathogen can persist in that population. If so, for a country to become free of an infection, also eradication of the pathogen in the wildlife species should take place.
Persistence can occur either locally or globally. Local persistence means that the pathogen can persist in an isolated population. This population does not need to have any contacts with other populations. If the pathogen can persist locally then it can also persist globally. Some pathogens however may persist only globally, which means that the pathogen can only persist in a meta-population, which is a set of partially isolated populations. These populations are able to exchange individuals through which transmission of the pathogen from one local population to another, where the pathogen became extinct, may occur.
Persistence can be expressed as the critical community size (CCS) that is required for persistence of the pathogen. The first example of determining a CCS is given by Bartlett (1957), who showed, using empirical data, that measles can persist in a meta-population of more than 250,000 individuals. However the CCS can not always be estimated (e.g. for frequency-dependent transmission) and therefore the persistence is also expressed as the mean time to extinction of a pathogen in a population.
Whether a pathogen may persist in a wildlife population depends on several factors. In the first place on population characteristics such as population size and turnover rate: the larger the population and the higher the turnover rate, the larger the probability of persistence. Second, there are other factors, depending on the host-pathogen relationship, which may enhance the probability that persistence does occur. Some of these factors will now be discussed. A distinction can be made between microparasites (prions, viruses, fungi, bacteria and protozoa) and macroparasites (nematodes, cestodes, trematodes, ticks and lice). Macroparasites have a higher probability that persistence occurs, compared to microparasites, because of: i) the many adaptations that allow helminth and arthropod larval stages to live for long periods of time in a dormant state; ii) the long periods of time

macroparasites can live in one animal; or iii) the complex multi-host life cycles that have evolved to allow parasites to use different host species at different times of the year, or in different parts of their hosts habitat (Hudson & Dobson, 1995 and references therein).
However, the focus of this thesis will be on microparasites. Also microparasites use various mechanisms and/or conditions to enable persistence such as spread by vertical transmission (e.g. retroviruses (in germline), classical swine fever, phocine distemper virus, baculoviruses and louping ill virus), survival in carriers (e.g. rabies, foot and mouth disease, bovine herpesvirus 1), durable spores (e.g. anthrax), long periods of infectiousness (e.g. brucellosis and tuberculosis), various reservoirs and alternative hosts (e.g. louping ill) (Dobson & Hudson, 1995 and references therein).

For studying the persistence of an infectious pathogen in a wildlife population information is needed about which geographic area(s), population(s) and wildlife species are involved in the transmission of the pathogen. Size and density of the population(s) of all involved wildlife species and their turnover rate(s) need to be estimated as well as how population size varies over time and how the turnover rate varies for different population sizes. The relation between population size and turnover rate is not always straightforward in wildlife populations. In wildlife populations, density-dependent effects can dominate recruitment, such that the replenishment rate of new susceptible individuals might decrease as the population size increases (Lloyd-Smith et al., 2005).
The gathered data may then be used as input for stochastic epidemic models. Using these models, the mean time to extinction can be estimated as it may not be possible to observe this in the field. For some infectious pathogens it may be possible to observe extinction of the pathogen in the field. In those cases, systematic monitoring and surveillance of the wildlife species may bring information about whether there is a pattern of recurrent epidemics without evidence of introduction of the pathogen from outside the observed population. When this happens there is a reason to believe that the pathogen can persist. Note, in-between epidemics it can be difficult to detect whether or not the pathogen has become extinct or is present at a low endemic level.

After having explored whether the infectious pathogen can persist when taking only the population characteristics into account, other factors, depending on the host-pathogen relationship, should also be incorporated in the stochastic epidemic model. However, it may not be easy to observe for example whether carrier individuals exist or whether vertical transmission occurs in the wildlife population. At first it can be assumed that the same host-pathogen dynamics as in domestic animal populations can also occur in the taxonomically

related wildlife species. More detailed data on the wildlife population should be gathered for generating hypotheses for the host-pathogen relationship that may make persistence of the pathogen possible. For example information about age, gender, contact structure, clinical signs, and the biotic and abiotic environment of the host and of the pathogen is needed. The isolation of the infectious pathogen from the animal and from the environment could give an idea about possible transmission routes and infectious periods. Thereafter, detailed experiments and epidemic models can be used to further test the hypothesis.

Eradication and control strategies

If a pathogen can persist in a wildlife population then for a country to become free of the disease, eradication of the pathogen also has to take place in the wildlife population. Especially when the risk at possible transmission from the wildlife species to other populations is very small, the decision to eradicate a pathogen in the wildlife species depends on various considerations. This decision will not only depend on technical considerations, such as the possibility of vaccination or stamping out, but also on political and ethical considerations.

If eradication is not feasible then it can be tried to control the infection. Strategies for eradication or control can be taken in both wildlife and domestic animal populations. Both types of strategies are meant to reduce the number of susceptible individuals in the population. Examples of such strategies are vaccination, population size reduction/selective removal of infected or susceptible individuals, breeding of disease resistant populations, separation of infected and susceptible individuals, environmental manipulation, fencing, disinfection etcetera (Wobeser, 2000 and references therein). Which control strategies to be taken will depend, among others, on the ecology of the disease, the animal species in question, the opinions of conservationists, farmers, and government, and the costs of the control measurements.

The focus in this thesis is on eradication and control strategies in wildlife populations. Some examples of control strategies that have been used in other projects related to wildlife populations are presented here. Culling of brushtail possums as a source of tuberculosis infection for cattle in New Zealand is the longest-running example of non-selective culling (Wobeser, 2000 and references therein). Another example of extensive culling of wild animals in order to reduce or eliminate *Mycobacterium bovis* in a wild population is the culling of over 20,000 badgers between 1975 and 1997, which was part of the British bovine tuberculosis (TB) control policy (Donnely et al., 2003).

The first example of pathogen elimination from a wild animal population by means of

vaccination and not by means of reduction of a host population was by Baer et al. (1971) who found that red foxes could be protected against rabies by means of oral vaccination with attenuated rabies viruses. Vaccination of foxes against rabies started in The Netherlands in 1988 and was stopped in 1991 after successful eradication of rabies (Vitasek, 2004).
In Germany a conventional live virus-vaccine based on the attenuated classical swine fever (CSF) virus strain 'C' is used for oral immunisation against CSF in wild boar. Results of oral vaccination experiments showed that oral vaccination has not been effective enough to achieve eradication of the virus in the total German wild boar population (Laddomada, 2000). In some cases intensive hunting of young boars was a necessary adjunct to the use of oral vaccination (Kaden et al., 2000).
Another example of vaccination is the oral delivery of a lipid-formulated *Mycobacterium bovis* bacilli Calmette-Guerin (BCG) vaccine to possums. This vaccine has been shown to be an efficient means of inducing protection against bovine tuberculosis in this wild type species and should be considered a practical way of vaccinating also other wildlife against bovine tuberculosis (Aldwell et al., 2003; Buddle et al., 2006).
When dealing with the threat of certain endemic diseases in Africa such as foot and mouth disease, African swine fever or theileriosis, containment has repeatedly shown to give the best results (Bengis et al., 2002). This option consists of control zones/areas, game-proof fences, cordons and movement control, which separate wildlife from domestic animals.
Other techniques that have been used in controlling outbreaks in wildlife, for example anthrax outbreaks, included burning/burying of carcasses, field burning, waterhole disinfection and remote vaccination by means of disposable darts or bio-bullets (Bengis et al., 2002).

Case study and objectives of this thesis

This thesis is about the role of Heck cattle (Van Vuure, 2005) and red deer (*Cervus elaphus*) for the possible introduction of bovine herpesvirus 1 (BHV1) in domestic cattle. BHV1 is a candidate to be eradicated among domestic cattle. Although the compulsory eradication programme for BHV1 in domestic cattle populations is suspended since February 1999, eradication of BHV1 still takes place on a voluntary basis (Dutch Animal Health Service). Farmers thus try to become certified for BHV1-free cattle and do not want their domestic cattle to become infected again by BHV1-infected feral animals. The possible persistence of BHV1 in feral animals is therefore an issue for those farmers. Thus the specific research question became whether BHV1 could persist in feral Heck cattle and red deer populations. Although persistence of BHV1 in feral animal populations does not necessarily imply a risk at BHV1 infection for domestic animals it is an important condition for such a risk to occur.

In The Netherlands, cattle farmers have to comply with several European and/or national rules for keeping animals in order to minimise the spread of specific pathogens. For example, cattle farmers have to identify and register their animals, surveillance of their cattle for certain diseases has to take place, and vaccination against specific pathogens has to be implemented. Feral animals in nature reserves may also be susceptible to infection with these pathogens. However, these rules do not have to be implemented in the feral animal populations in nature reserves to the extent as in domestic cattle populations. Conservationists in their strive for self sustaining nature, want to intervene in these feral animal populations as little as possible. As a result of the less stringent rules for keeping animals in nature reserves as compared to keeping cattle at farms, there is an ongoing debate between farmer organisations, conservationists and government about whether the health status of feral cattle jeopardises the health status of domestic cattle.
In this respect, eradication of bovine herpesvirus 1 (BHV1) is the most prominent acute problem. As serological surveys have indicated that various feral animal populations living in nature reserves in The Netherlands are also infected with BHV1 (Dutch Animal Health Service; Van Essen and Van Leeuwen, 1997), the question arises whether the BHV1-infected feral animal populations are a threat for the eradication of BHV1 in domestic cattle populations. These feral animal populations are: approximately 600 Heck cattle in 'the Oostvaardersplassen' (OVP), approximately 1000 red deer in OVP, approximately 130 Heck cattle in 'Slikken van Flakkee' (SFL) and approximately 139 Heck cattle in 'Hellegatsplaten' (HPL). The population of Scottish Highlanders in 'Veluwe Zoom' appears to be free of the infection (Dutch Animal Health Service; Van Essen and Van Leeuwen, 1997).
BHV1 is an alphaherpesvirus that infects cattle causing infectious bovine rhinotracheitis and genital infections (Gibbs and Rweyemamu, 1977). Once individuals are infected with a herpesvirus they remain carriers of the virus for life (Gibbs and Rweyemamu, 1977; Ackermann et al., 1982). Moreover, under certain stress conditions the virus can reactivate and carrier hosts become infectious again (Ackermann et al., 1982; Sheffy and Rodman, 1973; Dennett et al., 1976; Hage, 1997; reviewed by Jones, 2003). Carrier hosts may then establish primary infections in susceptible animals (Hage, 1997).

Questions have been asked at the minister of agriculture, nature and food quality about the possibility of BHV1 introduction from OVP as a result of an isolated case of BHV1 introduction at a domestic certified BHV1-free cattle farm at 5 km distance from OVP. In a correspondence with the parliament (23 October 1998 MKG. 983395) the same minister has answered that it was not very likely that the BHV1 introduction was from OVP. The minister also has said that future policy will be directed at excluding that feral cattle in nature reserves will be a significant

threat to the BHV1 eradication programme in domestic cattle. In order to be able to answer the question whether infected Heck cattle populations will be a threat for the eradication of BHV1 in domestic cattle populations a study was started on the dynamics of BHV1 in the three above mentioned Heck cattle populations. From several studies (Bosch, 1997; Hage, 1997; Mars, 2000) it is known that domestic cattle are a core group for BHV1. We also know from serological data that Heck cattle can be infected with BHV1. It is very likely that also the Heck cattle are a core group for BHV1 as the Heck cattle are a crossbred from various domestic cattle breeds. Thus, if Heck cattle are a core group for BHV1 then BHV1 may also persist in this population. From a study of De Koeijer (2003) we know that the lifelong infection with BHV1 causes population persistence of BHV1 in local cattle populations. However, the stochastic model of De Koeijer (2003) did only account for the stochasticity due to reactivation and not for stochasticity due to other effects. In chapter 2 of this thesis the mean time to extinction for various population sizes is estimated using a model, which is a fully stochastic extension of the model of De Koeijer (2003). In chapter 3 the contact structure of part of the Heck cattle population in OVP is quantified using behaviour observations of animals. This was done to study how the contact structure would influence BHV1 transmission and thus also BHV1 persistence. Then in chapter 4 the transmission of BHV1 among farmed red deer under experimental conditions is quantified. For the eradication of BHV1 in cattle, it is important to know whether red deer alone can play a significant role in the transmission of BHV1. In chapter 5 the effects of vaccination, as one of the eradication strategies, are studied on the dynamics and persistence of BHV1 in the Heck cattle populations. For this study serological data of BHV1 in the Heck cattle populations were combined with model simulations of the dynamics of a BHV1 infection. In chapter 6 the most important findings in the previous four chapters and other options for eradication of BHV1 in large feral Heck cattle populations were discussed. Besides, it was also discussed whether eradication of BHV1 should take place in the Heck cattle population in OVP.

References

Ackermann, M., Peterhans, E., Wyler, R., 1982. DNA of bovine herpesvirus type 1 in the trigeminal ganglia of latently infected calves. Am. J. Vet. Res. 43, 36-40.

Aldwell, F.E., Keen, D.L., Parlane, N.A., Skinner, M.A., De Lisle, G.W., Buddle, B.M. 2003. Oral vaccination with *Mycobacterium bovis* BCG in a lipid formulation induces resistance to pulmonary tuberculosis in brushtail possums. Vaccine 22, 70-76.

Baer, G.M., Abelseth, M.K., Debbie, J.G. (1971). Oral vaccination of foxes against rabies. Am. J. Epidemiol. 93, 487-490.

Bartlett, M.S. 1957. Measles periodicity and community size. J.R. Stat. Soc. A 120, 48-70.

Becker, N.G. 1989. Analysis of infectious disease data. Chapman and Hall, New York.

Bengis, R.G., Kock, R.A., Fisher, J. 2002. Infectious animal diseases: the wildlife/livestock interface. Rev. Sci. Tech. Off. Int. Epiz. 21, 53-65.

Bosch, J.C. 1997. In: Bovine herpesvirus 1 marker vaccines: tools for eradication? PhD-thesis. University of Utrecht, The Netherlands.

Bouma, A. 1997. Transmission of pseudorabies virus: an experimental approach. PhD-thesis. University of Utrecht, The Netherlands.

Buddle, B.M., Wedlock, D.N., Denis, M. 2006. Progress in the development of tuberculosis vaccines for cattle and wildlife. Vet. Microbiol. 112, 191-200.

Dennett, D.P., Barasa, J.O., Johnson, R.H., 1976. Infectious bovine rhinotracheitis virus: studies on venereal carrier status in range cattle. Res. Vet. Sci. 20, 70-83.

Diekmann, O., Heesterbeek, J.A.P., Metz, J.A.J., 1990. On the definition and computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J. Math. Biol. 28, 365-382.

Dobson, A.P., Hudson, P.J. 1995. Microparasites: Observed patterns in wild animal populations. In: Grenfell, B.T. & Dobson, A.P. (eds.), Ecology infectious diseases in natural populations. Cambridge University Press, Cambridge, 52-89.

Donnelly, C.A., Woodroffe, R., Cox, D.R., Bourne, J., Gettinby, G., Le Fevre, A.M., Mclnerney, J.P., Morrison, W.I. 2003. Impact of localized badger culling on tuberculosis incidence in British cattle. Nature 426, 834-837.

Dutch Animal Health Service (Gezondheidsdienst voor Dieren), Deventer, The Netherlands (http://www.gd-dieren.nl).

Van Essen, G.J., Van Leeuwen, J.M. 1997. Gezondheidsaspecten van grote grazers in natuurgebieden. ID-DLO rapport 98.005.

Garnett, B.T., Delahay, R.J., Roper, T.J. 2002. Use of cattle farm resources by badgers (*Meles meles*) and risk of bovine tuberculosis (*Mycobacterium bovis*) transmission to cattle. Proc. R. Soc. Lond. B 269, 1487-1491.

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpesviruses, Part I, Bovine herpesvirus 1. Vet. Bull. 47, 317-343.

Greenwood, M., Bradford Hill, A.T., Wilson, W.W.C. 1936. Experimental epidemiology. London, UK: HM Stationary Office.

Hage, J.J. 1997. In: Epidemiology of bovine herpesvirus 1 infections. PhD-thesis. University of Utrecht, The Netherlands.

Hudson, P.J., Dobson, A.P. 1995. Macroparasites: Observed patterns in animal populations. In: Grenfell, B.T. & Dobson, A.P. (eds), Ecology infectious diseases in natural populations. Cambridge University Press, Cambridge, 144-176.

Jones, C. 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. Clin. Microbiol. Rev. 16, 79-95.

De Jong, M.C.M., Kimman, T.G. 1994. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 12, 761-766.

Kaden, V., Lange, E., Fischer, U., Strebelow, G. 2000. Oral immunisation of wild boar against classical swine fever: evaluation of the first field study in Germany. Vet. Microbiol. 73, 239-252.

Kermack, W.O., Mckendrick, A.G. 1936. Contributions to the mathematical theory of epidemics IV. Analysis of experimental epidemics of mouse typhoid: a bacterial disease conferring incomplete immunity. J. Hyg. 39, 271-288.

De Koeijer, A.A. 2003. In: Infectious diseases among animals: combining models and data. PhD-thesis. University of Utrecht, The Netherlands.

Laddomada, A. (2000). Incidence and control of CSF in wild boar in Europe. Vet. Microbiol. 73, 121-130.

Lloyd-Smith, J.O., Cross, P.C., Briggs, C.J., Daugherty, M., Getz, W.M., Latto, J., Sanchez, M.S., Smith, A.B., Swei, A. 2005. Should we expect population thresholds for wildlife disease? TRENDS in Ecology and Evolution, 20, 511-519.

Mars, M.H. 2000. In: Vaccinology and epidemiology of bovine herpesvirus 1 infections. PhD-thesis. University of Utrecht, The Netherlands.

Sheffy, B.E., Rodman, S., 1973. Activation of latent infectious bovine rhinotracheitis virus infection. J. Am. Vet. Med. Assoc. 163, 850-851.

Vitasek, J. 2004. A review of rabies elimination in Europe. Vet. Med. 49, 171-185.

Van Vuure, C. (ed.), 2005. Retracing the Aurochs: history, morphology and ecology of an extinct wild ox. Pensoft, Sofia-Moscow, pp 431.

Wobeser, G. 2000. Disease management strategies for wildlife. Rev. Sci. Tech. Off. Int. Epiz. 21, 159-178.


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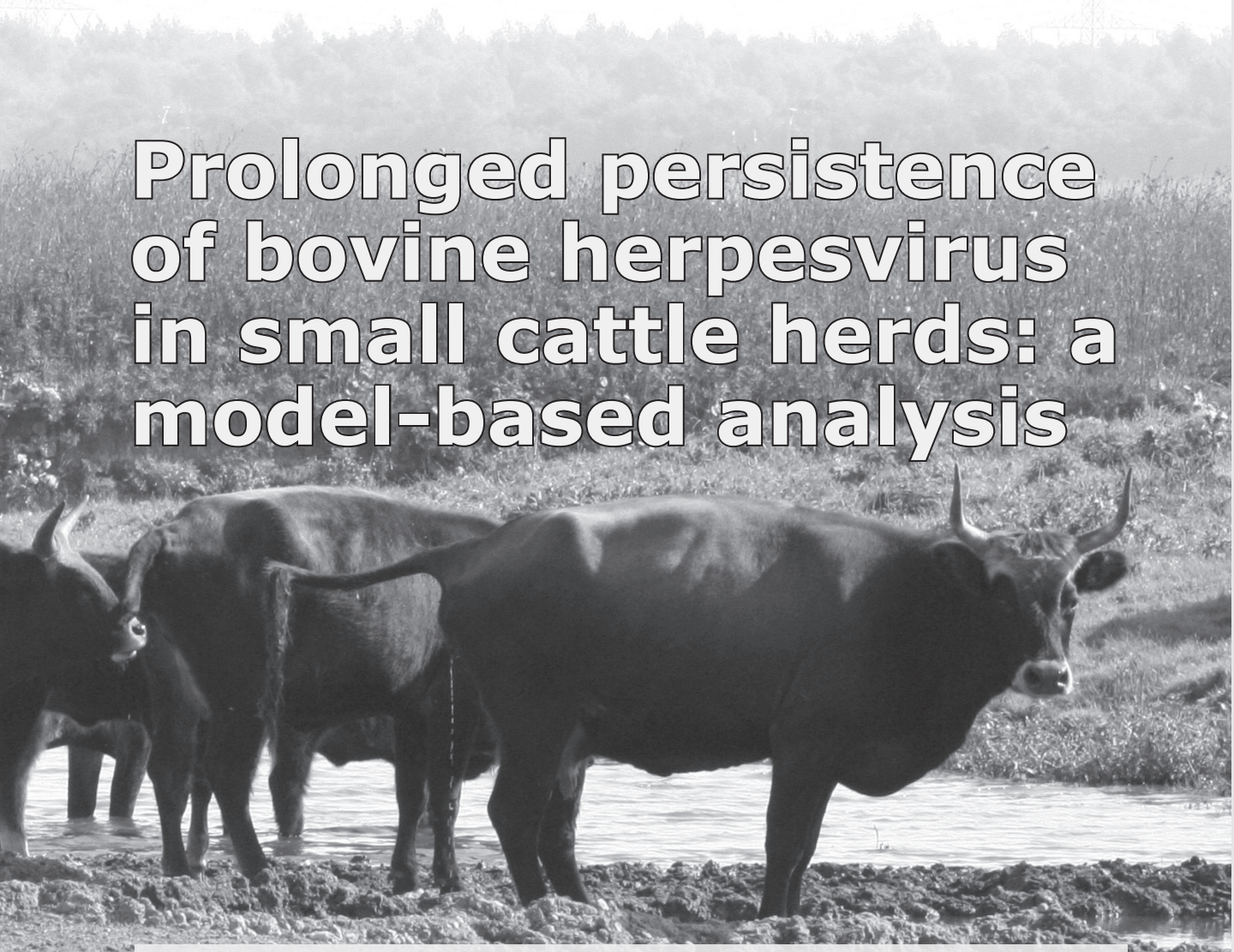
CHAPTER 2

Prolonged persistence of bovine herpesvirus in small cattle herds: a model-based analysis



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Summary

Herpesviruses can remain dormant in once-infected hosts and, upon reactivation, cause such hosts to become infectious. This phenomenon of latency and reactivation may enable herpesviruses to persist for a long time in small host populations. To quantify the effect of reactivation on persistence, the time to extinction of bovine herpesvirus type 1 (BHV-1) in small cattle populations was calculated. For realistic parameter values the mean time to extinction is already more than 100 years in a population of 10 animals. In a population of 20 animals the mean time to extinction is approximately 2000 years. The effects of vaccination on persistence were also studied, revealing that continued vaccination of the whole population could result in much faster eradication. For instance, in an isolated herd of 20 animals BHV-1 could be eradicated in 44 years.

Introduction

Extinction of an infectious pathogen in any finite local host population is certain and has been observed and modelled (Bartlett, 1957; Bartlett, 1960; Grenfell, 1992; Keeling, 1997). The time to extinction of an infectious pathogen is dependent on its host-pathogen relationship. Measles, of which the extinction events have been well documented, cannot persist beyond the duration of a single epidemic even within fairly large local populations (<250 000 individuals) (Bartlett, 1957). In a meta-population context like the cities of England and Wales (Grenfell and Bolker, 1998), no extinction of measles was observed in the troughs between epidemics. This was probably due to a re-introduction of measles from one local population with measles, to another local population where measles has already become extinct.

The reason why the persistence of herpesviruses (e.g. bovine herpesvirus type 1 (BHV-1), equine herpesvirus type 1, Marek's disease virus, varicella-zoster virus) is very different from the persistence of measles is because herpesviruses possess properties that enable them to survive in small host populations for a long time. Once individuals are infected with a herpesvirus they remain carriers of the virus for life (Ackermann et al., 1982; Gibbs and Rweyemamu, 1977) and, under certain stress conditions, the virus can reactivate and the carrier hosts become infectious again (Ackermann et al., 1982; Dennett et al., 1976; Hage et al., 1996; Sheffy and Rodman, 1973).

Recently De Koeijer (2003) developed a model for calculating the time to extinction of herpesviruses, which they subsequently applied to BHV-1 in cattle. Importantly, the model analysis necessitated a separation into two time-scales: 1) a short time-scale during which the infection and recovery processes take place and 2) a long time-scale during which the reactivation and birth events take place. This separation into short and long time-scales was possible because the infection and recovery processes occur on a much faster time-scale than the birth and reactivation processes. For instance, the time between infection and recovery of BHV-1 in cattle is approximately 1 week, whereas the lifespan of cattle and the time between reactivation events of BHV-1 in cattle is in the order of years. However, De Koeijer's model (2003) does not account for all stochastic effects of the dynamics of BHV-1 in cattle. In particular, in the model: 1) only major outbreaks were taken into account, while minor outbreaks were ignored; 2) no stochasticity in the size of the outbreak was incorporated; and 3) stochasticity in the birth-death process was omitted, using a deterministic description of the host demography.

Yet, we believe that incorporation of the above stochastic effects may be vital to obtain more realistic calculations of the time to extinction in small populations. Here we studied

the impact of demographic stochasticity and stochasticity in the size of the outbreak on the time to extinction of BHV-1. The dynamics were modelled using a fully stochastic extension of the model of De Koeijer (2003). For the analysis of the model and its variants we used analytical results available on Markov chain models where possible. Those variants that could not be formulated as standard Markov models were studied by simulation. We studied the implications for management directed at eradication of BHV-1 within local populations, especially the effect on the time to extinction of population size and of vaccination.

Model structure and analysis

Model overview

Two separate time-scales were considered: 1) a short time-scale (days or weeks) during which infection and recovery events take place; and 2) a long time-scale (years) during which birth, death and reactivation events take place. Separation of the two time-scales can be safely done if the birth, death and reactivation rates are small compared to the infection and recovery rates. In essence, we assumed that epidemic outbreaks take place instantaneously on the long time-scale. This assumption greatly simplified the model as it kept the number of events small, and it enabled us to describe the dynamics of the long time-scale solely by the number of latently infected individuals (i.e. individuals that have become carriers of the virus without being infectious). The dynamics of our model are governed by a discrete-time Markov chain. Hence, the probability of a population being in a particular state $m(t)$ on day t, conditional on it being in state $k(t\text{-}1)$ on the previous day t-1, was: 1) independent of the population's behaviour prior to day t-1; and 2) dependent only on the value $k(t\text{-}1)$ and not on t explicitly. The short time-scale was modelled by focusing on the probability distribution of outbreak sizes. Subsequently, the distribution of the outbreak sizes was incorporated into the long time-scale during which birth, death and reactivation events took place.

The short time-scale: outbreaks

We first considered the short time-scale during which outbreaks occur after a reactivation event of a latently infected individual. In the following, $S(t)$ denotes the number of susceptible individuals at time t, $I(t)$ denotes the number of infected and infectious individuals at time t, and $P(t)$ denotes the number of latently infected individuals at time t. Throughout, total population size is denoted by N and was assumed to be constant (i.e., $N=N(t)=S(t)+I(t)+P(t)$). Thus, the population state during the short time-scale can be denoted by the pair $(I(t), P(t))$, whereas the population state during the long time-scale is determined by $P(t)$ only, as infectious individuals are absent during inter-epidemic periods.

An outbreak starts with a reactivation event after which the population has amongst it a single infected and infectious individual. This infectious individual may infect a number of susceptible individuals, who in turn may infect other susceptible individuals. The outbreak ends when the infection chain has stopped, namely, when the number of infectious or susceptible individuals has dropped to zero. Figure 1 gives a schematic structure of the possible routes that the infection chain can take.

By standard arguments, it was assumed that susceptible individuals are infected at a rate, $\beta \frac{I}{N}$ where β (time^{-1}) is the transmission rate constant. Infected individuals recover from infection at a rate α (time^{-1}), so that $\frac{1}{\alpha}$ corresponds to the infectious period.

Note that the above model formulation entails the following assumptions: 1) all infectious individuals are equally infectious; 2) all susceptible individuals are equally susceptible; 3) each infected individual poses an identical and independent risk of infection to each susceptible individual; and 4) the transmission rate parameter and the recovery rate are constant over time.

Given the above assumptions, the probability that an infection event occurs before a recovery event occurs is given by the infection rate $\beta \frac{SI}{N}$ divided by the sum of the infection rate and the recovery rate $\beta \frac{SI}{N} + \alpha I$. Hence, the probability that an infection event will occur before a recovery event is given by: $\frac{R_1 S}{R_1 S + N}$, where $R_1 = \frac{\beta}{\alpha}$. Likewise, the probability that a recovery event occurs before an infection event is given by $\frac{N}{R_1 S + N}$, which is the recovery rate αI divided by the sum of the infection rate and the recovery rate. The parameter R_1 represents the reproduction ratio of a single outbreak, namely, the number of newly infected individuals infected by one infectious individual during one infectious period in a fully susceptible population. Note, the reproduction ratio R_1 does not depend on the population size *N* (De Jong and Kimman, 1994). Using the above formulations we can calculate the probability distribution of the final size of an outbreak (Ball, 1986; Kroese and De Jong, 2001). The final size gives the probability distribution of the number of initially susceptible individuals that have been infected and have become latently infected (*P*) at the end of the outbreak.

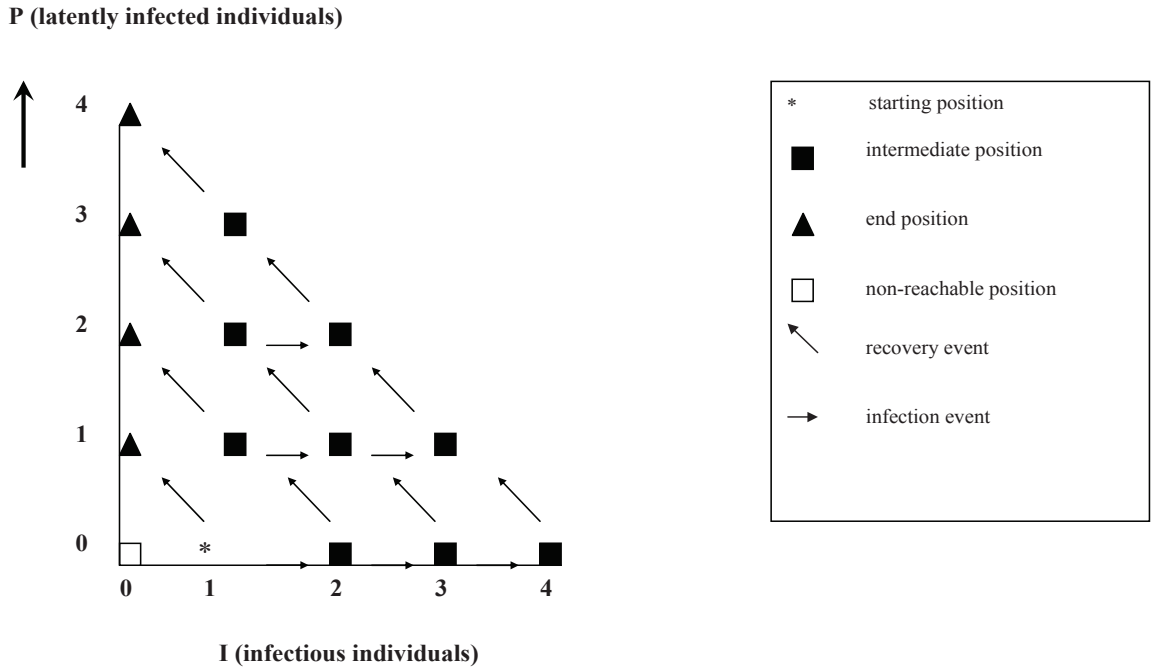

Fig. 1. Possible routes of the infection chain in a population of four animals. The population state is given by the number of infectious animals, *I*, and the number of latently infected animals, *P*. Each population state is a vertex of the grid in the (*I*,*P*)-plane. Starting from the vertex, marked by an asterisk, the population state will jump from one vertex to another until it reaches one of the absorbing states where $I = 0$. Vertexes on this axis are the final population states, i.e. the states where there are no more infectious individuals (Ball, 1986; Kroese and de Jong, 2001).

The long time-scale: demographic turnover and reactivation

On the long time-scale there are no infectious individuals and three types of events may occur: birth, death and reactivation. Our assumption that population size *N* remains constant requires that birth and death events are coupled so that a deceased individual is immediately replaced by a newborn susceptible individual. Birth-death events occur at a rate μ per individual. Thus, the total birth-death rate is given by μN. In practise only the death of a latently infected individual is of importance because the death of a susceptible individual results in an identical susceptible individual.

A seropositive latently infected individual reactivates at a rate *v*. Hence, the total reactivation rate is given by *vP*. De Koeijer (2003) showed that the number of reactivation events per host lifetime is crucial to the time to extinction. The number of reactivation events of a latently infected individual during its lifetime is given by the geometric series

$$\frac{\mu}{\mu+\nu}\sum_{i=0}^{\infty} i\left(\frac{\nu}{\nu+\mu}\right)^{i} = \frac{\nu}{\mu}$$

As explained in the previous section, a latently infected individual that re-excretes virus causes an outbreak, the size of which may vary. In the following we will denote by the element f_{ij} the probability that the population contains *i* latently infected individuals before a reactivation event, while it contains *j* latently infected individuals after the event. The outbreak size *j – i* depends on the parameter values of the infection process (α and β), and on the number of susceptible individuals at the start of the outbreak *(S(t))*.

After having described the dynamics on the short and long time-scale we are now able to determine the overall reproduction ratio, R_0, for a reactivating virus, which is defined as the number of newly infected individuals infected by one infectious individual during its lifetime in a fully susceptible population. The overall reproduction ratio (R_0) is equal to the reproduction ratio of a single outbreak (R_1) plus the expected number of times reactivation events take place per host lifetime ($\frac{\nu}{\mu}$) times the reproduction ratio of a single outbreak (R_1): $R_0 = (1 + \frac{\nu}{\mu})R_1$. As a consequence it is possible that $R_1 < 1$ while $R_0 > 1$. This will happen whenever the reactivation rate ν is high relative to the mortality rate μ.

Analysis of the model

With the Markov model at hand, several interesting properties such as the mean time to extinction can be calculated. The transition matrix, **M**, containing the transition probabilities on the long time-scale can be partitioned so that a matrix **Q** contains only the entries corresponding to the transient states. Then direct application of standard Markov chain theory teaches us that the so-called fundamental matrix **K** is given by $\mathbf{K} = (\mathbf{I} - \mathbf{Q})^{-1}$ (Kemeny and Snell, 1960), where **I** denotes the identity matrix.

If the initial distribution over the non-absorbing states is given by a row vector **r**, then the mean time to extinction *E[t]* is given by

$$E[t] = \mathbf{r}.\mathbf{K}.\mathbf{1}, \quad (1)$$

where **1** represents the vectors of ones. Likewise the variance of the time to extinction *Var[t]* is given by

$$Var[t] = \mathbf{r}.(2\mathbf{K}-\mathbf{I}).\mathbf{K}.\mathbf{1}-(\mathbf{r}.\mathbf{K}.\mathbf{1})^2. \quad (2)$$

Illustration

To illustrate how the short and the long time-scales were integrated we present a specific example in which the total population contains four individuals *(N = 4)*. On the short time-scale, a 4x4 matrix **F** contains the probability distribution of outbreak sizes (equation 3). This probability distribution of outbreak sizes is then subsequently incorporated into the 5x5

transition matrix **M**, which describes the long time-scale (equation (4)). For simplicity, the time-step Δt in the matrix **M** is set at 1. The two matrices take the following form

$$\mathbf{F} = \begin{pmatrix} \frac{4}{4+3R_1} & \frac{12R_1}{(2+R_1)^2(4+3R_1)} & \frac{96{R_1}^2(8+3R_1)}{(2+R_1)^2(4+R_1)^3(4+3R_1)} & \frac{3{R_1}^3(160+R_1(96+R_1(16+R_1)))}{(2+R_1)^2(4+R_1)^3(4+3R_1)} \\ 0 & \frac{2}{2+R_1} & \frac{16R_1}{(2+R_1)(4+R_1)^2} & \frac{{R_1}^2(8+R_1)}{(2+R_1)(4+R_1)^2} \\ 0 & 0 & \frac{4}{4+R_1} & \frac{R_1}{4+R_1} \\ 0 & 0 & 0 & 1 \end{pmatrix} \quad (3)$$

$$\mathbf{M} = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ \mu & 1-\mu-\nu(1-f_{11}) & \nu f_{12} & \nu f_{13} & \nu f_{14} \\ 0 & 2\mu & 1-2\mu-2\nu(1-f_{22}) & 2\nu f_{23} & 2\nu f_{24} \\ 0 & 0 & 3\mu & 1-3\mu-3\nu(1-f_{33}) & 3\nu f_{34} \\ 0 & 0 & 0 & 4\mu & 1-4\mu \end{pmatrix} \quad (4)$$

The dynamics are determined by the matrix equation

$$\mathbf{x}(t+1) = \mathbf{x}(t).\mathbf{M} \quad (5)$$

where **x** is a (row) vector containing the distribution of latently infected individuals over the various population states. The elements f_{ij} ($1 \le i,j \le N$) and m_{ij} ($0 \le i,j \le N$) of the transition matrices **F** and **M** represent the probabilities that the population contains i latently infected individuals before an event, while it contains j latently infected individuals after the event. Note that the indices i and j run from 1 to 4 in **F** and from 0 to 4 in **M**.

Simulation model

We also developed a simulation model to investigate the robustness of the results of the Markov model and to examine the impact of the assumption that the host lifespan is exponentially distributed. To this end we extended the model by considering: 1) a fixed host lifespan; and 2) an exponentially distributed host lifespan with fixed maximum age (i.e., a truncated exponentially distributed host lifespan). For this comparison all simulations started with only latently infected individuals ($N = 20$). Apart from different assumptions on the distribution of the host lifespan, the simulation model contained the same processes as the Markov model. Per parameter combination 1000 replicates were taken. The simulations were stopped when no latently infected individuals were left in the host population. The time-step in the model was chosen such that the probability at two events occurring at the same time was approximately 0.01.

Parameter values

Parameter values were derived from data of both feral and domestic cattle populations. The mean host lifespan was estimated from demographic data of the Heck cattle population in the Dutch nature reserve 'De Oostvaardersplassen' (Cornelissen and Vulink, 1996; Platteeuw et al., 1998; Platteeuw et al., 1999; Platteeuw et al., 2000). Because there were no data available on the dynamics of BHV-1 within feral cattle populations, data from field studies were taken describing the dynamics of BHV-1 (Bosch, 1997) within domestic dairy cattle herds in The Netherlands. The reproduction ratio of a single outbreak of BHV-1 was estimated at 3.2 (Bosch, 1997). The reproduction ratio of a single outbreak under vaccination conditions was set at 0.45. The reactivation rate was calculated by De Koeijer (2003) from data of field studies done by Bosch (1997) and was estimated at 0.09 per year. The same value for the reactivation rate under vaccination conditions was used (De Koeijer, 2003). For the population size we referred again back to the Heck cattle population. The Heck cattle population is a structured population. Various social units were distinguished in the Heck cattle population: 1) solitary animals; 2) bull groups; 3) mixed groups; and 4) cow groups. Mature bulls often stayed in small groups (2-30 animals), while cow groups could contain larger numbers of animals (20-100) (Vulink, 2001). These group sizes varied during the year. The population size in this study was set at 20 individuals (range 2-50), referring to the Heck cattle population of 'De Oostvaardersplassen'. As the initial condition, we took the expected distribution belonging to the case in which the virus was already present in the population for a relatively long time (i.e. technically this distribution corresponds to the quasi-stationary distribution (Caswell, 2001; Diekmann and Heesterbeek, 2000). The initial population state vector was given by the quasi-stationary distribution with $R_1 = 3.2$. In a sense, the quasi-stationary distribution

Table 1: Default values and the range of parameters in the Markov model and simulation model*.

Parameter	Default value (range)	Ref.
Population size (N)	20 (2 - 50)	(Vulink, 2001)
Mortality rate (μ)	0.1 year^{-1} (0.1 – 0.5)	(Cornelissen and Vulink, 1996; Platteeuw et al., 1998; Platteeuw et al., 1999; Platteeuw et al., 2000)
Reactivation rate (ν)	0.09 year^{-1} (0 – 0.5)	(De Koeijer, 2003)
Reproduction ratio of a single outbreak (R_1)	3.2 (0.45 - 50)	(Bosch, 1997)

* Data refers to a Heckcattle population in the Dutch nature reserve 'De Oostvaardersplassen' and to data of domestic dairy cattle herds in The Netherlands.

corresponds to a worst-case scenario. Table 1 shows the default values and the range of values considered.

Results

Default parameter setting

First, we considered the fate of the pathogen in a small population ($N = 20$) in which initially one infectious individual is present while all remaining individuals are susceptible. Motivated by empirical data (Bosch, 1997) we chose $R_1 = 3.2$ for the reproduction ratio of a single outbreak. Other parameters were as shown in Table 1. Figure 2 shows the results. Figure 2a gives the probability distribution just after the first outbreak. The probability distribution is markedly bimodal with peaks at $P = 1$ and at $P = 20$. A reactivation event in a latently infected individual resulted in a minor outbreak in approximately 35% of the cases in which only a minority of the susceptible individuals (say $1 < P < 8$) is infected. On the other hand, once a certain critical number of susceptible individuals have been infected, the remaining susceptible individuals are unlikely to escape infection. In fact, the probability that all susceptible individuals are infected (i.e. $P = 20$ after the outbreak) is approximately 25%.

Figure 2b-e shows the probability distributions after 1, 10, 100, and 1000 years. Figure 2b illustrates that the probability of extinction of the pathogen after 1 year is just 2%. The most likely outcome is that the population contains one latently infected individual ($P = 1$) while the remaining individuals are susceptible. As time progresses the probability of extinction increases gradually, so that after 1000 years the probability of extinction is approximately 50%.

In case the pathogen has not become extinct after 1000 years, it is highly likely that 10-20 latently infected individuals are present (Fig. 2e). This is because once the population contains predominantly latently infected individuals it will take a very long time before all latently infected individuals have died in the population conditional on no new outbreaks having taken place. Roughly speaking the right-hand-sided peak in Figure 2 corresponds to the so-called quasi-stationary distribution. Even after 1000 years it is still highly probable that the population has not yet reached the absorbing state. In fact, with a probability of 0.48 the population contains predominantly latently infected individuals ($11 < P < 19$).

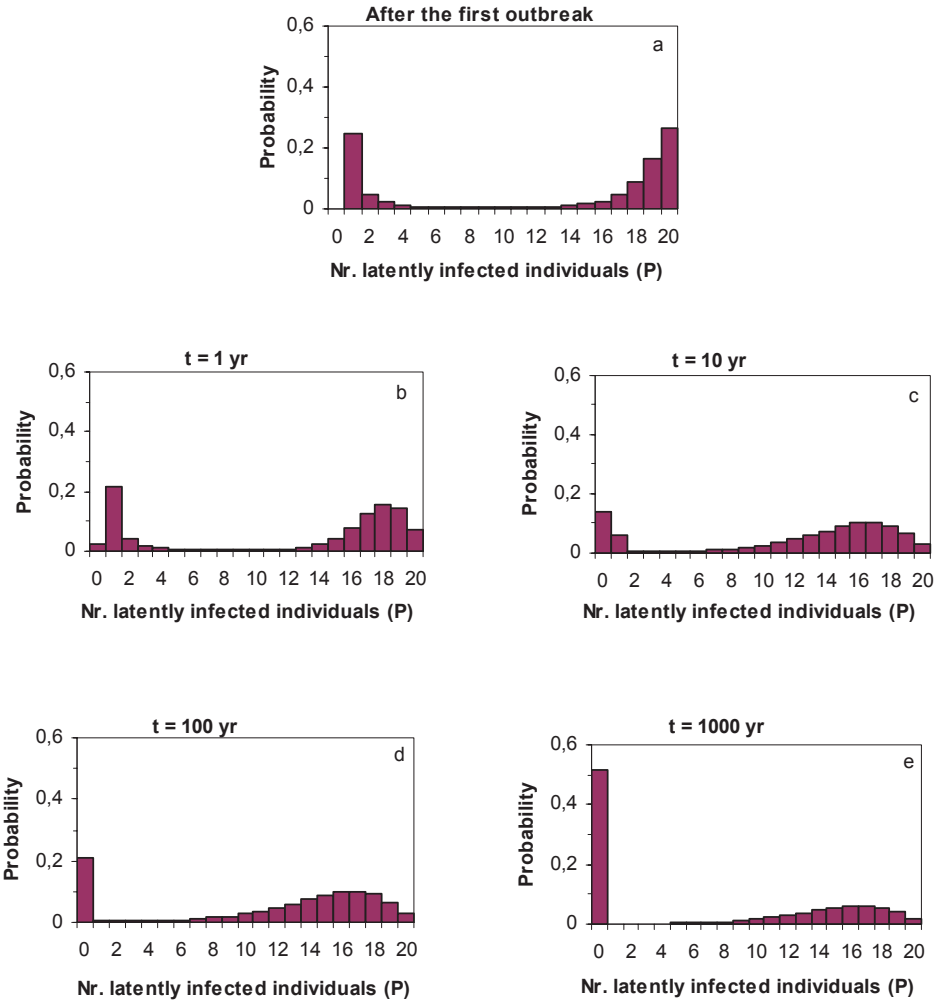

Fig.2. Probability distribution of the number of latently infected individuals (*P*) in a population after (a) introduction of one infectious individual; (b) 1 year; (c) 10 years; (d) 100 years; and (e) 1000 years. The total population size is set at $N = 20$. The number of latently infected individuals at the x-axis ranges from 0 to 20.

Initial conditions

To study the effect of the initial conditions on the time to extinction we considered three scenarios: 1) one individual is latently infected and the remaining individuals are susceptible; 2) one individual is infectious and the remaining individuals are susceptible; and 3) the population distribution corresponds to the quasi-stationary distribution. Parameter values are as in Table 1, and Figure 3 shows the results.

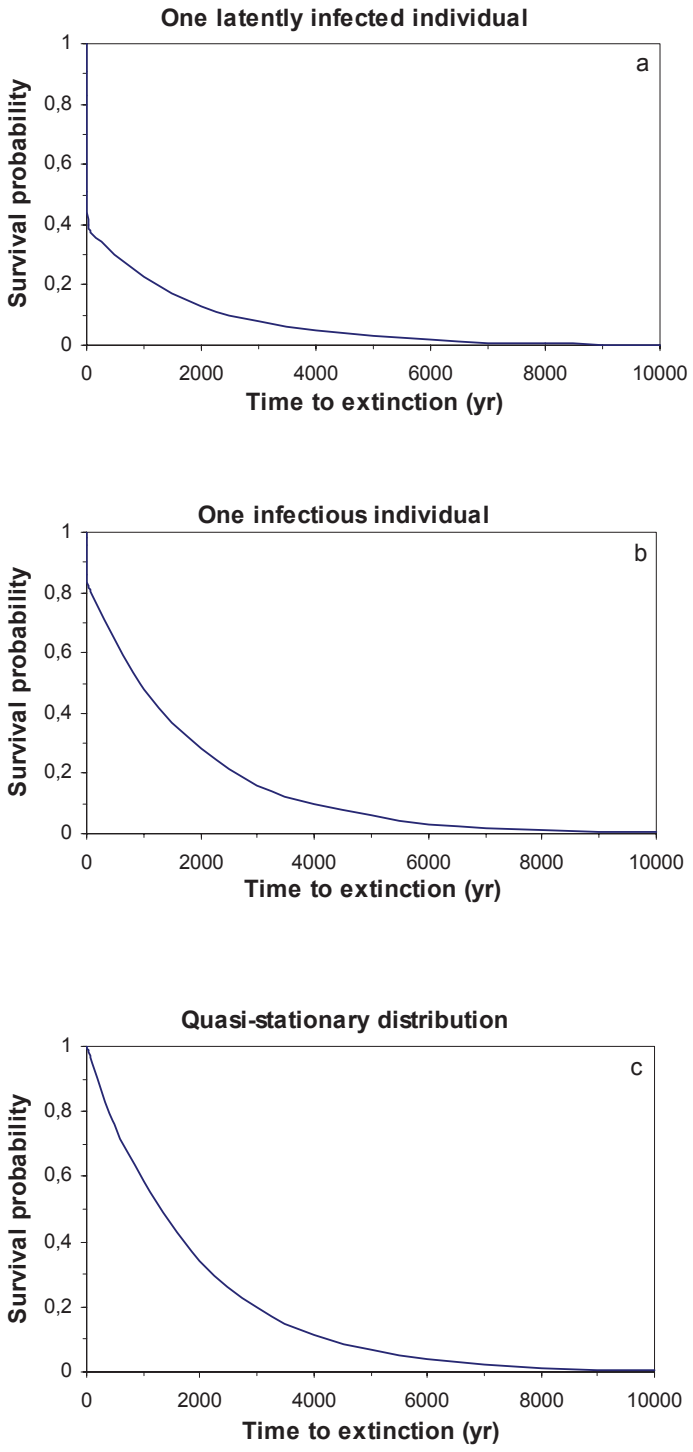

Fig.3. The time to extinction against the survival probability of BHV-1 within a population of size N =20. We considered three different initial distributions: (a) one individual is latently infected and the remaining individuals are susceptible; (b) one individual is infectious and the remaining individuals are susceptible; and (c) the population distribution corresponds to the quasi-stationary distribution.

As illustrated in Figure 3a, if the population contains one latently infected individual, the pathogen is quickly (within 10 years) driven to extinction with a probability of 0.49. On the other hand if the pathogen does not become extinct within this time-span, it may persist for a very long time (> 10 000 years). The intuitive explanation is that the pathogen will become extinct in a short space of time only if the latently infected individual dies before a reactivation event takes place. If, on the other hand, a reactivation event leading to a major outbreak takes place before the latently infected individual dies, the population will contain mainly or exclusively latently infected individuals and extinction of the pathogen may take a very long time.

If initially a single infectious individual is present in the population, the probability of extinction within a short time-span decreases considerably. In fact, the probability of rapid extinction (within 10 years) is just 14%. The intuitive explanation is that there will be an immediate outbreak if an infectious individual is introduced.

Figure 3c shows the results of a case where, initially, the probability distribution over the population states is given by the quasi-stationary distribution. Here, it is very unlikely that the pathogen becomes extinct in a short time-span, as it is unlikely that the population has only one or a few latently infected individuals.

Population size

The impact of an increase in the population size *(N)* on the time to extinction is illustrated in Figure 4. Figure 4a,b refers to two different values of the reproduction ratio of a single outbreak (R_1), one well above the critical value 1 ($R_1 = 3.2$) and one well below 1 ($R_1 = 0.45$). In both cases R_0 exceeds 1. Figure 4c refers to the situation where both $R_1 < 1$ and $R_0 < 1$. In Figure 4a the quasi-stationary distribution with $R_1 = 3.2$ was taken as initial distribution and in Figure 4b,c the quasi-stationary distribution with respectively $R_1 = 3.2$ (smoothed line) and $R_1 = 0.45$ (dashed line) were taken as initial distributions.

If both $R_1 > 1$ and $R_0 > 1$ (Fig. 4a), then the mean time to extinction increases exponentially with increasing N. Even in relatively small populations the time to extinction may be high (e.g. 126 years if $N = 10$). In larger populations (e.g. $N = 50$) the mean time to extinction is in the order of millions of years.

If $R_1 < 1$ and $R_0 > 1$ (Fig. 4b), the time to extinction increases more or less exponentially for relatively large population sizes ($N > 20$) and increases less than exponentially for values of $N < 20$. Note, the initial distribution is of marginal importance for the time to extinction.

If both $R_1 < 1$ and $R_0 < 1$ (Fig. 4c), then the time to extinction increases less than exponentially for all values of N. The time to extinction increases marginally if N is large. Intuitively, this can be understood as follows. If $R_0 < 1$ an infectious individual will infect only a few susceptible

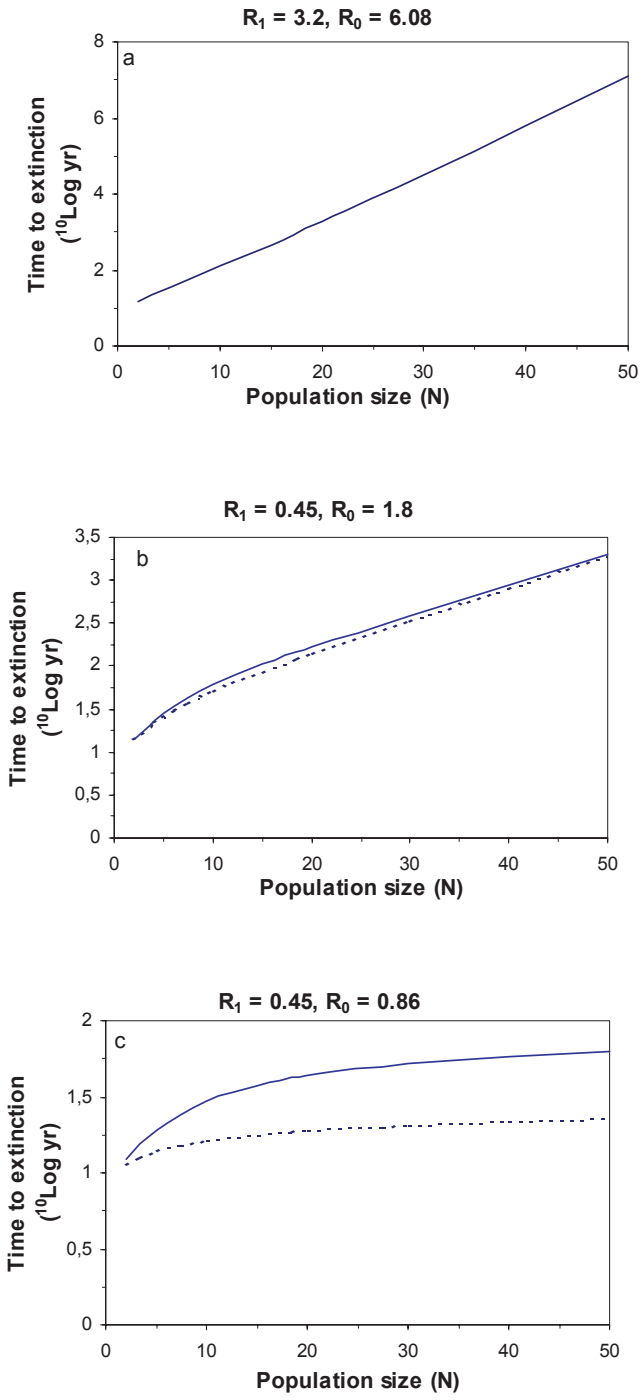

Fig.4. The mean time to extinction as a function of population size (N) and three different scenarios for the reproduction ratios. (a) Both $R_1 > 1$ and $R_0 > 1$; (b) $R_1 < 1$, $R_0 > 1$ and $\nu = 0.3$ year^{-1}; (c) both $R_1 < 1$ and $R_0 < 1$. In (a) the quasi-stationary distribution with $R_1 = 3.2$ was taken as the initial distribution and in (b) and (c) the quasi-stationary distribution with respectively $R_1 = 3.2$ (smoothed line) and $R_1 = 0.45$ (dashed line) were taken as initial distributions.

individuals. As a consequence the time to extinction is hardly affected anymore by population size. Note, the initial distribution is of importance now for the time to extinction.

Number of reactivation events per host lifetime

The effect of changing the number of reactivation events per host lifetime is illustrated in Figure 5. Population size N was fixed at $N = 20$, and the lifespan of the host was kept constant at 10 years. The reactivation rate was varied systematically from 0-0.5 (year^{-1}), corresponding to 0 to 5 reactivation events per host lifetime. This implies that the overall reproduction ratio

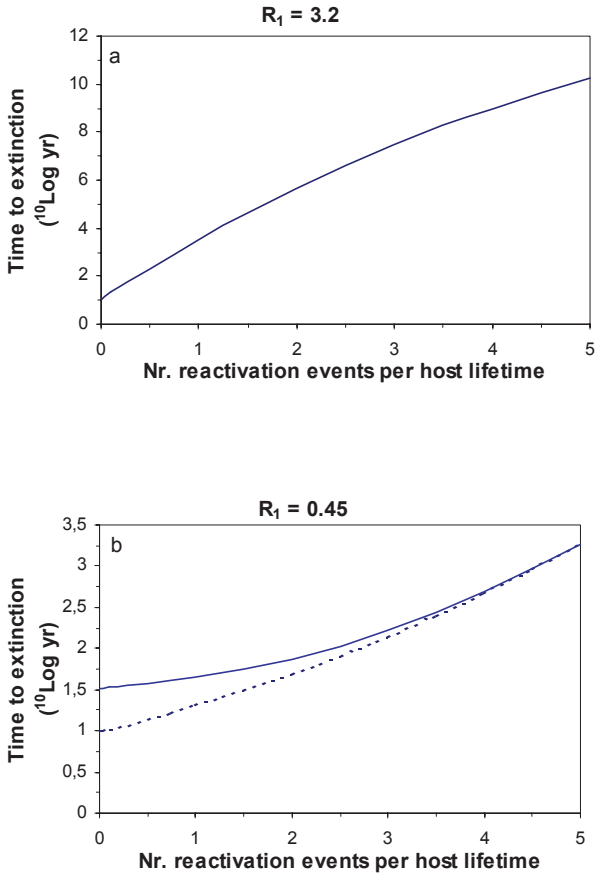

Fig. 5. The mean time to extinction as a function of the number of reactivation events per host lifetime $\left(\frac{\nu}{\mu}\right)$ and two different values of the reproduction ratio of a single outbreak (R_1). Note in (a) R_1 is 3.2 and (b) R_1 is 0.45. In (a) the quasi-stationary distribution with R_1 = 3.2 was taken as initial distribution and in (b) the quasi-stationary distribution with respectively R_1 = 3.2 (smoothed line) and R_1 = 0.45 (dashed line) were taken as initial distributions.

R_0 varies from R_0 = 3.2 to R_0 = 19.2 if R_1 = 3.2 (Fig. 5a), and from R_0 = 0.45 to R_0 = 2.7 if R_1 = 0.45 (Fig. 5b). In Figure 5a the quasi-stationary distribution with R_1 =3.2 was taken as initial distribution and in Figure 5b the quasi-stationary distribution with respectively R_1 = 3.2 (smoothed line) and R_1 = 0.45 (dashed line) were taken as initial distributions.

The figures show that the time to extinction increases with an increasing number of reactivation events per host lifetime. If $R_1 > 1$ (Fig. 5a) then the time to extinction increases less than exponentially whereas if $R_1 < 1$ (Fig. 5b) the time to extinction increases faster than exponentially. Thus the pathogen might still persist for a long time if the expected number of reactivation events per host lifetime is sufficiently large (> 3) to bring R_0 sufficiently above 1.

The reproduction ratio of a single outbreak R_1

The effect of changing R_1 on the mean time to extinction is studied and illustrated in Figure 6. The quasi-stationary distribution accompanying each value of R_1 was taken as the initial distribution. The figure shows that the time to extinction increases less than exponentially if R_1 increases. The impact on the time to extinction is larger for values of $R_1 < 10$ than for values of $R_1 > 10$. The time to extinction reaches an asymptote for large values of R_1. Intuitively, this can be understood as follows. For relatively large values of R_1 the probability of a major outbreak goes to 1 and thus all susceptible individuals in the population will already be infected.

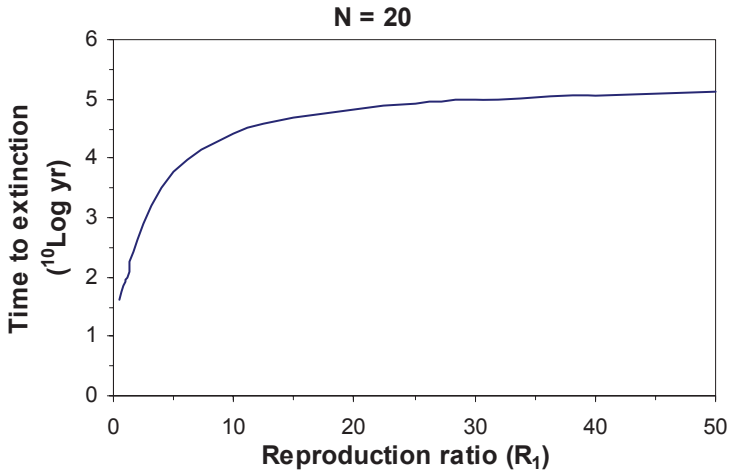

Fig. 6. The mean time to extinction as a function of the reproduction ratio of a single outbreak (R_1). The quasi-stationary distribution accompanying each value of R_1 was taken as the initial distribution. Other parameters were set at their default values.

The distribution of the host lifespan

The simulation model allows us to explore the impact of various assumptions on the distribution of the host lifespan.

Specifically we considered: a) a fixed host lifespan; and b) a truncated exponentially distributed host lifespan. To be able to make a fair comparison, the mean host lifespan was kept constant at 10 years in all scenarios. In the case of a fixed host lifespan each individual lives exactly 10 years. In the case of a truncated exponentially distributed host lifespan, the mortality rate was set at 0.05 $year^{-1}$ and the maximum age at 14 years. Table 2 shows the times to extinction in the case of an exponentially distributed host lifespan versus a fixed host lifespan for different values of R_1. For all values of R_1 the time to extinction is lower in a model with a fixed host lifespan than in a model with an exponentially distributed host lifespan. Intuitively this can be explained as follows. In the case of a fixed host lifespan all individuals live exactly 10 years whereas in the case of an exponentially distributed host lifespan some individuals live for a very short time and some individuals live relatively long. For those individuals that live relatively long there still remains the probability of a reactivation event during the time that the population contains latently infected individuals. In the case of a truncated exponentially distributed host lifespan and relatively small or large values of R_1, the time to extinction lays in between the values for the time to extinction in the case of an exponentially distributed host lifespan and a fixed host lifespan.

Table 2: The times to extinction in years (S.E.) in the case of an exponentially distributed host lifespan and in the case of a fixed host lifespan and for five different values of R_1*.

R_1	**Exponentially distributed host lifespan**	**Fixed host lifespan**
0.45	48.16 (0.85)	20.41 (0.35)
1.1	104.57 (1.44)	74.67 (2.67)
1.5	212.33 (5.16)	140.67 (4.81)
2.0	449.08 (12.96)	229.06 (4.13)
3.2	1899.56 (67.28)	492.24 (17.38)

* Three host lifetime distributions are compared with each other, namely an exponentially distributed host lifetime, a fixed host lifetime and exponentially distributed host lifetime with a maximum age. The mean time to extinction in years is given for the first two host lifetime distributions.

Demographic stochasticity and stochasticity in the size of the outbreak

To study the effect of demographic stochasticity and stochasticity in the size of the outbreak we compared our model, which included both types of stochasticity with the model of De Koeijer (2003), which did not include those types of stochasticity. Their analysis was based on the following assumptions: 1) only large outbreaks were taken into account, while small outbreaks were ignored; 2) outbreaks could only occur when the fraction of susceptible individuals reached a critical fraction (x_0) at which $R_1 > 1$; 3) the probability of a major outbreak was approximated by ($1-\frac{1}{xR_1}$)(where x is the fraction of susceptible individuals); and 4) stochasticity in the birth-death process was omitted. In our more realistic model with finite population we did not make an artificial distinction between major and minor outbreaks. For technical reasons, the time to extinction in this section was calculated as the time until the last outbreak had taken place.

First, the impact of stochasticity in the size of the outbreak on the time to extinction was studied. Figure 7 shows the results. For a reproduction ratio of a single outbreak just above 1, the mean time to extinction in our model (smoothed line) was substantially larger compared to the model of De Koeijer (2003) (dashed line), as is shown in Figure 7a. In our model, with an exponentially distributed infectious period, the probability of a minor outbreak is given by the inverse of the reproduction ratio of a single outbreak, assuming the density of the susceptible individuals is 1. For instance if $R_1 = 1.5$ the probability of a minor outbreak is given by $\frac{1}{1.5} = 0.67$. For relatively small values of the reproduction ratio (R_1) the probability of a minor outbreak becomes larger. For larger values of the reproduction ratio ($R_1 > 3$) our results were similar to the results of De Koeijer (2003), as is shown in Figure 7b. Hence, we conclude that minor outbreaks can not be ignored for values of R_1 close to 1.

Second, we systematically studied the impact of the host lifespan and the reproduction ratio of a single outbreak on the time to extinction. Figure 8 shows the results. In short, the analysis showed that for values of R_1 near to or just above 1 the mean times to extinction were larger for reasons explained in the previous paragraph. For large values of $R_{1,}$ on the other hand, the time to extinction in ref. (De Koeijer, 2003) may be considerably larger than in our model. The intuitive reason is that in ref. (De Koeijer, 2003) the fraction of latently infected individuals could reach very small values close to zero at which point major outbreaks could still take place, whereas in our model the last latently infected individual would already have died by chance.

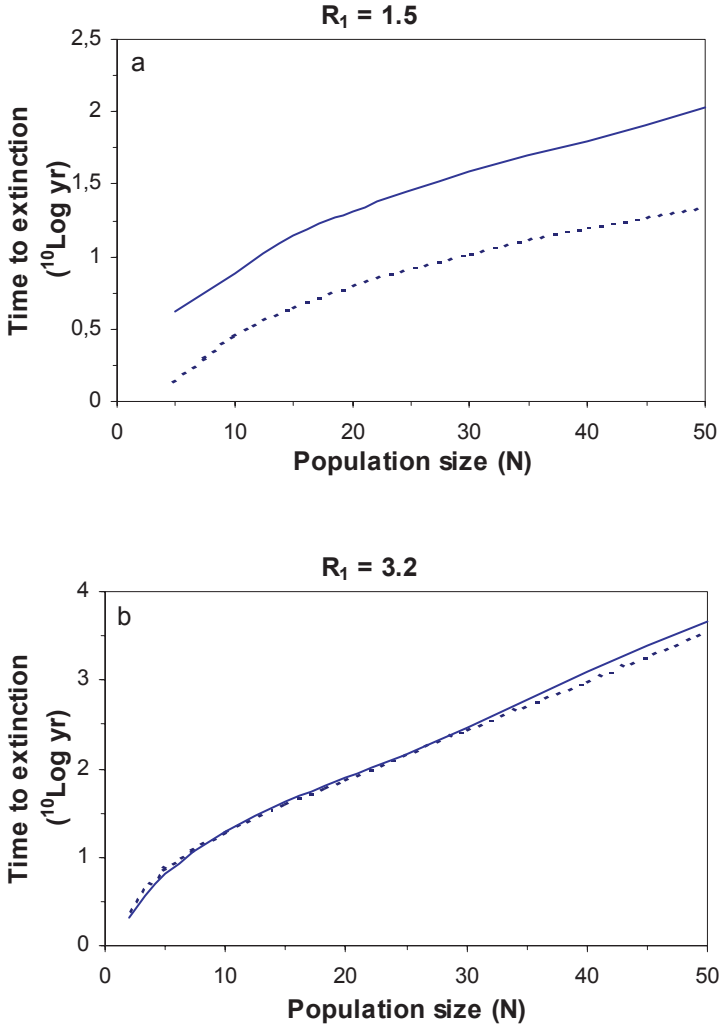

Fig. 7. The mean time to extinction determined with our model (smoothed line) compared with the model of De Koeijer (2003) (dashed line) as a function of the population size. Note in (a) R_1 is 1.5 and in (b) R_1 is 3.2. The host lifespan was set at 5 years and other parameters were set at their default values. In both models we started with a number of susceptible individuals (S) equal to the critical density (x_0) times the population size N, and $N - S$ latently infected individuals (P).

Discussion

Compared to other viruses, herpesviruses have an eye-catching mechanism, which may enable them to survive for a long time in small populations. They have the possibility of reactivation after recovery of the host, which may have profound consequences for the eradication of the virus.

In this paper we calculated the time to extinction for BHV-1 in small closed cattle populations using a Markov model that takes into account demographic stochasticity and stochasticity in

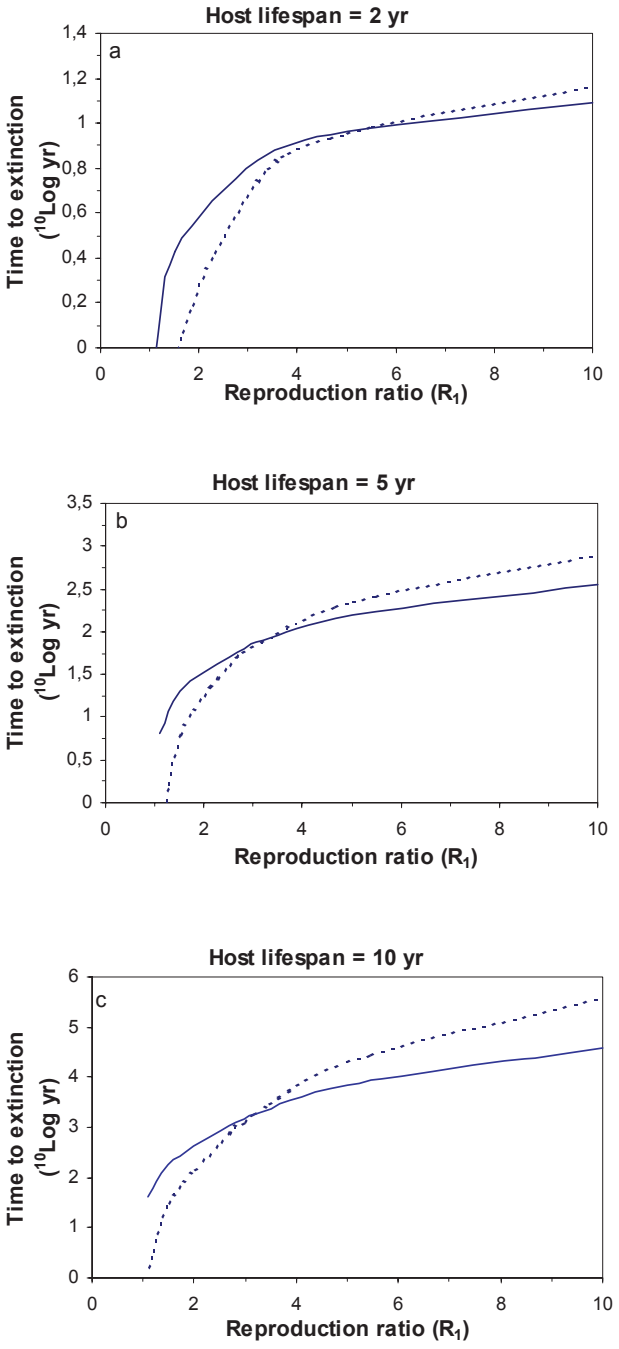

Fig.8. The mean time to extinction determined with our model (smoothed line) compared with the model of De Koeijer (2003) (dashed line) as a function of the reproduction ratio of a single outbreak (R_1). We considered three different values for the host lifespan namely, 2 years, 5 years and 10 years. In both models we started with a number of susceptible individuals (S) equal to the critical density (x_0) times the population size N, and $N - S$ latently infected individuals (P).

the size of an outbreak. Specifically, we examined the impact of the population size, mortality rate, reactivation rate, reproduction ratio of a single outbreak and the overall reproduction ratio on the time to extinction.

Our results indicate that for realistic parameter values the mean time to extinction is already in the order of hundred years in small populations ($N = 10$). In larger populations (e.g., $N = 50$) the mean time to extinction increases strongly, and can be in the order of millions of years. In fact, our results indicated that a relatively short time to extinction (say in the order of 60 years) can only be achieved if both R_1 and R_0 are below 1. Given the demography of the Heckcattle population this implies that the reactivation rate has to be relatively low ($\nu < 0.1$ year^{-1}).

A reproduction ratio R_1 smaller than 1 might be achieved by vaccinating a sufficient part of the population. Vaccination might be a useful tool to achieve eradication of BHV-1. Suppose, for instance, that vaccines were available that were able to reduce the reproduction ratio of a single outbreak (R_1). If, hypothetically, by vaccination R_1 dropped from 3.2 to 0.45 then for a population of 50 animals the mean time to extinction decreases from several millions of years to approximately 60 years. For a population of 100 individuals the time to extinction becomes approximately 80 years and for a population of 1000 individuals the time to extinction becomes 150 years. For practical purposes this is however still a very long time.

Alternatively, vaccination could result in a decrease in the number of reactivation events per host lifetime. In fact, there is evidence that this can be achieved by: 1) vaccinating susceptible individuals with a gE-negative BHV-1 vaccine strain (Mars, 2000) or a latency-related (LR) mutant of BHV-1 (Inman et al., 2002); or 2) by reducing the host lifespan of latently infected individuals. For sufficiently small values of the reactivation rate ($\nu = 0.01$ year^{-1}) and $R_1 = 3.2$ the time to extinction can be decreased to 50 years even in a population of 50 animals.

Prior to 1998 BHV-1 infections in cattle were widespread in The Netherlands. For instance, a BHV-1 bulk milk survey in 1994 revealed that at least 84% of the dairy herds had seropositive cattle (Van Wuijckhuise et al., 1998), while on average 12% of these herds had seropositive young stock (Van Wuijckhuise et al., 1998). This led the Dutch authorities to introduce an integrated eradication campaign in 1998. From 1997 to 2000 the seroprevalence of milking cows in The Netherlands had decreased strongly (from 40% to 22%) as a result of the integrated eradication campaign. At the same time the total number of BHV-1-free certified herds had increased from 3000 herds in 1997 to almost 16 000 herds in 2000 (Dutch Animal Health Service). During the eradication campaign, the purchase of cattle to complement a certified BHV-1-free herd was only permitted from other certified BHV-1-free herds. All cattle over 3 months of age in herds not proved to be BHV-1-free had to be vaccinated twice a year.

For feral cattle, on the other hand, intervention measures such as vaccination may not be achievable. Furthermore the lifespan of feral cattle may be at least twice as long as that of domestic cattle, and population sizes can also be much larger. We have shown that a longer mean lifespan and a larger population size both increase the time to extinction to such an extent that for practical purposes the virus will persist indefinitely. To what extent circulation of BHV-1 in feral cattle possess a risk to commercial farms remains to be investigated.

Acknowledgements

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References

Ackermann, M., Peterhans, E., Wyler, R., 1982. DNA of bovine herpesvirus type 1 in the trigeminal ganglia of latently infected calves. Am. J. Vet. Res. 43, 36-40.

Ball, F., 1986. A unified approach to the distribution of total size and total area under the trajectory of infectives in epidemic models. Adv. Appl. Prob. 18, 289-310.

Bartlett, M.S., 1957. Measles periodicity and community size. J. R. Stat. Soc. A 120, 48-70.

Bartlett, M.S., 1960. The critical community size for measles in the U.S. J. R. Stat. Soc. A 123, 37-44.

Bosch, J.C., 1997. In: Bovine herpesvirus 1 marker vaccines: tools for eradication? Ph.D. Thesis. University of Utrecht, The Netherlands.

Caswell, H., 2001. In: Matrix Population Models. Sunderland: Sinauer Associates, 56-109.

Cornelissen, P., Vulink, J.Th., 1996. Large herbivores in wetlands: evaluation of the grazing regime in "De Oostvaardersplassen". Flevobericht 399, pp 142 [in Dutch].

Dennett, D.P., Barasa, J.O., Johnson, R.H., 1976. Infectious bovine rhinotracheitis virus: studies on venereal carrier status in range cattle. Res. Vet. Sci. 20, 70-83.

Diekmann, O., Heesterbeek, J.A.P., 2000. In: Mathematical epidemiology of infectious diseases: model building, analysis and interpretation. Chichester: Wiley, 73-95.

Dutch Animal Health Service (Gezondheidsdienst voor Dieren), Deventer, The Netherlands. (http://www.gd-dieren.nl).

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpesviruses, Part I, Bovine herpesvirus 1. Vet. Bull. 47, 317-43.

Grenfell, B.T., 1992. Chance and chaos in measles dynamics. J.R. Stat. Soc. B 54, 383-98.

Grenfell, B.T., Bolker, B.M., 1998. Cities and villages: infection hierarchies in a measles population. Ecol. Lett. 1, 63-70.

Hage, J.J., Schukken, Y.H., Barkema, H.W., Benedictus, G., Rijsewijk, F.A.M., Wentink, G.H., 1996. Population dynamics of bovine herpesvirus 1 infection in a dairy herd. Vet. Microbiol. 53, 169-80.

Inman, M., Lovato, L., Doster, A., Jones, C., 2002. A mutation in the latency-related gene of bovine herpesvirus 1 disrupts the latency reactivation cycle in calves. J. Virol. 76, 6771-6779.

De Jong, M.C.M., Kimman, T.G., 1994. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 12, 761-6.

Keeling, M.J., 1997. Modelling the persistence of measles. Trends Microbiol. 5, 513-18.

Kemeny, J.G., Snell, J.L., 1960. In: Finite Markov Chains. New Yersey: D. Van Nostrand, pp. 43-58.

De Koeijer, A.A., 2003. In: infectious diseases among animals: combining models and data. Ph.D. Thesis. University of Utrecht, The Netherlands.

Kroese, A.H., De Jong, M.C.M., 2001. Design and analysis of transmission experiments. In: Menzies FD, Reid SWJ eds. Proc. of a meeting of the Soc. Vet. Epidemiol. Prev. Med. Noordwijkerhout: The Netherlands, xxi-xxxvii.

Mars, M.H., 2000. In: Vaccinology and Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis, University of Utrecht, The Netherlands, pp. 145.

Platteeuw, M., Cornelissen, P., Jans, L., 1998. Vegetation, grazing and birds in a fresh water swamp: Monitoring programme "De Oostvaardersplassen" 1996/1997. In: Report by the Institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands 98.096X, 35-49 [in Dutch].

Platteeuw, M., Jans, L., Cornelissen, P., Beemster, N., Altenburg, W., 1999. Vegetation, grazing and birds in a fresh water swamp: Monitoring programme "De Oostvaardersplassen" 1997/1998. In: Report by the Institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands 99.119X, 28-95 [in Dutch].

Platteeuw, M., Cornelissen, P., Beemster, N., Altenburg, W., Van Der Heide, Y., 2000. Vegetation, grazing and birds in a fresh water swamp: Monitoring programme "De Oostvaardersplassen" 1998/1999. In: Report by the Institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands 120X, pp. 109 [in Dutch].

Sheffy, B.E., Rodman, S., 1973. Activation of latent infectious bovine rhinotracheitis virus infection. J. Am. Vet. Med. Assoc. 163, 850-1.

Vulink, J.Th., 2001. In: Hungry Herds: Management of temperate lowland wetlands by grazing. Ph.D. Thesis, University of Groningen, The Netherlands.

Van Wuijckhuise, L., Bosch, J., Franken, P., Frankena, K., Elbers, A.R., 1998. Epidemiological characteristics of bovine herpesvirus 1 infections determined by bulk milk testing of all Dutch dairy herds. Vet. Rec. 142, 181-184.


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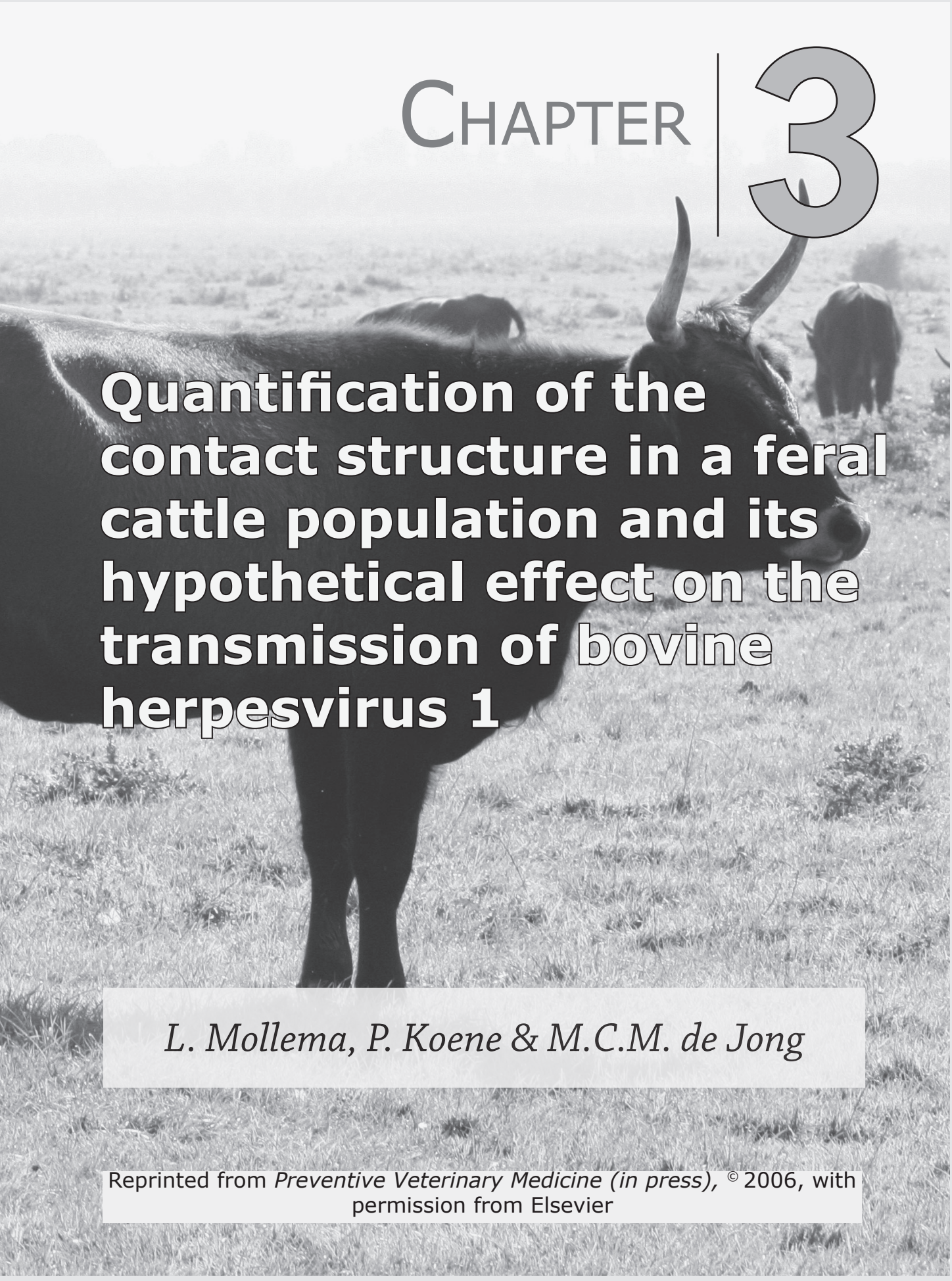

Chapter 3

Quantification of the contact structure in a feral cattle population and its hypothetical effect on the transmission of bovine herpesvirus 1

L. Mollema, P. Koene & M.C.M. de Jong

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Summary

The organisation of animal populations in social groupings may play a crucial role in the transmission of any infectious disease that requires close contact. The objective of this study was to quantify the contact structure of part of the Heck cattle population in a Dutch nature reserve and its hypothetical effect on the transmission of bovine herpesvirus 1 (BHV1). The contact structure was quantified by observing the number of different animals with whom contact was made (i.e. the number of contactees) within a fixed time period. Two types of behaviour sampling methods, namely focal sampling and scan sampling were used to observe the contact structure. In this study only those contacts between individuals were observed that were assumed to be a proxy measure of an at-risk event for BHV1-infection. Two reproduction ratios (*R*), i.e. the average number of new cases caused by a typical infected individual, were estimated, one for the observed contact structure and another for a random mixing contact structure. The two reproduction ratios were then compared to study the hypothetical effect on BHV1 transmission.

Results showed that the contact structure of the homogeneous population did differ significantly from a random mixing contact structure. The variation in the number of contactees was higher than under random mixing.

The overall number of contactees was highest during summer and lowest during winter-spring. Bulls, young bulls and cows had the highest number of contactees during respectively summer, autumn and winter-spring. From the analysis of the contingency tables it was clear that contacts between animal types did not occur at random during summer and autumn. For example, during summer more contacts than expected occurred between bulls and cows. We took this heterogeneity at animal type level into account in the calculation for *R*, which resulted for the observed contact structure in higher estimates for *R* than for the homogeneous population.

When looking at heterogeneity at individual level it was found that during summer almost all individuals were observed together direct or indirect in the same group except for certain bull groups. During autumn and winter-spring almost all individuals were seen together in the same group when considering a long contact period of fourteen days but the groups were fallen apart in smaller groups and solitary individuals for a short contact period of five days.

It could be concluded that based on the observed contact structure transmission would be favoured most during summer.

Introduction

Many animal populations, such as bovines, live in social groupings (Sinclair, 1977) for part of the year (Lazo, 1994). The organisation of animal populations in social groupings may play a crucial role in the transmission of any infectious disease that requires close contact. For example when social groupings live isolated from each other during time periods longer than the period of disease outbreaks, the epidemic may be limited to the group in which the infection is started.

The animal population in this study is a population of Heck cattle (Van Vuure, 2005) – a crossbred from various races resembling the extinct aurochs *Bos primigenius* – living in a nature reserve in The Netherlands. The social organisation of the Heck cattle population was studied by Vulink (2001) from June 1991 until July 1992. Vulink (2001) described four social groups in the Heck cattle population namely: i) solitary bulls; ii) bull groups; iii) cow groups; and iv) mixed groups. The home range and the home range size of mature bulls and cow groups were studied. Mature bulls often stayed in small groups and only used a restricted part of the area. Cow groups on the other hand, consisted of large numbers of animals and moved among the home ranges of various bull groups. The differences between mature bulls and cow groups in home range size were always distinct and the differences in home range varied during the year, being greatest in winter (Vulink, 2001).

We were then interested in the influence of the social organisation of the Heck cattle population on disease transmission. The pathogen bovine herpesvirus 1 (BHV1) was chosen for this study because most (110 out of 124) of the above mentioned Heck cattle, that were tested in the period 1996-2003, were seropositive for BHV1 using a gB blocking ELISA. BHV1 is an alphaherpesvirus that infects cattle causing infectious bovine rhinotracheitis (IBR) and genital infections (Gibbs and Rweyemamu, 1977). This study focused on the subtype of BHV1 that causes IBR. If a susceptible individual is infected, the infectious individual may excrete the virus over a period of between ten and seventeen days (Wentink et al., 1993). Once the virus has infected an individual it remains in that individual for life. An individual that is already infected with BHV1 is called a latently infected individual. Latently infected individuals may re-excrete the virus either spontaneously or under certain stress conditions (e.g. transport, parturition) but for a shorter time period than does a primary infectious individual (Wentink et al., 1993). Latently infected individuals that re-excrete virus may establish primary infections within susceptible animals in contact with the excreting animal. If not all latently infected individuals have died or have been taken out of that population before the infection is transmitted, BHV1 will persist in the population.

The objective of this study was to quantify the contact structure of part of the Heck cattle

population in a Dutch nature reserve and its hypothetical effect on BHV1 transmission. We started with the observation and analysis of the contact structure. One of the observations was the number of different animals with whom contact was made within a fixed time period. Heterogeneity at animal type level and at individual level were taking into account in the observation and analysis of the contact structure. Next, the method for calculating the potential for BHV1 transmission was described. The distribution of the number of different animals with whom contact was made, was used to calculate a reproduction ratio, which is defined as the average number of new cases caused by a typical infected individual (Diekmann et al., 1990). Reproduction ratios were calculated both for the observed contact structure and also for a random mixing contact structure. Random mixing was chosen as this type of mixing is predominantly used in epidemiological models. The two reproduction ratios were then compared to study the hypothetical effect of the actual observed contact structure on BHV1 transmission. This comparison was done because the absolute reproduction ratio could not be estimated. The hypothetical effects of the observed contact structure on BHV1 transmission and on the persistence of BHV1 were discussed.

Material and Methods

Observation of the contact structure

General methods

This study involved the herd of Heck cattle in the eastern part of the grazing area of in total 2000 ha in the nature reserve 'the Oostvaardersplassen' in The Netherlands. This part of the Heck cattle population lived most of the time separated from the Heck cattle population in the western part of the grazing area. The total number of Heck cattle was approximately 600 animals. The number of Heck cattle in the eastern part ranged from 310 – 340 animals. The counts were done in the summer period in 2001 and in the winter-spring period in 2002.

The study was divided into three study periods: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). The observation period was during daylight from 9 a.m. till 4 p.m. The Heck cattle were classified according to the following five animal types: i) cows (female > 2 years old); ii) bulls (male > 2 years old); iii) young cows (female between 7 months and 2 years old); iv) young bulls (male between 7 months and 2 years old); and v) calves (male and female < 7 months old). It was thought that those animal types could differ in their contact rates and mixing patterns.

A number of individuals were individually identified, denoted further as identified individuals. The identified individuals were chosen for their distinguishing features such as shape and

size of the horns, body colour and scars. These animals were given a name, their features were described and of each animal a photograph was taken.

Observation methods

The observed contacts consisted of: i) the type of contacts; ii) the number of different animals with whom contact was made per 20 minutes, denoted as the number of contactees per 20 minutes; and iii) the number of contacts with the same animal per 20 minutes, denoted as the number of contacts per contactee per 20 minutes. The observation time of 20 minutes was chosen for practical reasons. All types of contacts were observed where animals were in close proximity with each other and had some form of interaction (e.g. herding) together with all direct contacts (e.g. nose-to-nose, genital sniffing). This was done because Mars (2000) had shown experimentally that BHV1 transmission was still possible over a distance of at least four metres. All types of contacts were treated as events and it was assumed that all types of contacts gave a certain chance for BHV1 transmission. Table 1 gives a detailed description of the different types of contacts observed.

Two types of behaviour sampling methods, namely focal sampling and scan sampling were used to observe the contact structure. With focal sampling (Martin and Bateson, 1993) an individual (either identified or non-identified) was observed for 20 minutes and its contacts were recorded. Focal samples were meant to estimate the mean number of contactees and the mean number of contacts per contactee. During summer only non-identified individuals were individually observed, using the focal sampling method because no individuals were identified yet at the start of the summer period. The number of individuals of a certain animal type that was observed, was according to the total number of individuals of that animal type that was present in the Heck cattle population. Always the first and the third animal from a certain animal type, counted from the left, were observed when arriving at a group of animals.

During autumn 19 individuals (4 cows, 4 bulls, 4 young cows, 3 young bulls, 4 calves) and during winter-spring 16 individuals (4 cows, 5 bulls, 2 young cows, 1 young bull and 4 calves) were daily observed when possible for 20 minutes. Sometimes the observers failed to find an identified animal and no observations were available for that day. Additionally, as many non-identified individuals as possible were observed during the observation period. The method of observing non-identified individuals was the same as was described earlier for the summer period. During autumn the identified individuals were observed for a maximum of 27 days during an observation period of 43 days. During winter-spring the identified individuals were observed for a maximum of 34 days during an observation period of 71 days.

The daily observation of identified animals was used to be able to estimate later on the number of contactees for a longer observation time than 20 minutes, which corresponded more to the duration of BHV1 excretion. Actually, we were interested in the number of contactees during the duration of BHV1 excretion but for practical reasons, animals could not be observed for such long periods. The longer observation time corresponded to a maximum of 1.3 days during autumn and a maximum of 1.6 days during winter-spring.
With scan sampling (Martin and Bateson, 1993) the whole group of animals was rapidly scanned before and after a focal sample or only once if no focal sample was taken. Behaviour per individual within a group was noted. This is meant that all types of contacts (see Table 1) and behaviour as lying, walking, standing and grazing were observed. Scan samples were meant to estimate the proportion of time spent on making contacts and to observe which identified animals were seen together in the same group. Just before the end of the summer 49 animals (19 cows, 24 bulls, 3 young cows, and 3 young bulls) were identified. Note calves were not yet identified. These 49 identified individuals were observed for two continuous weeks at the end of the summer period using the scan sampling method. This was done to study which identified individuals were seen together direct or indirect in the same group within a fixed time period. Two or three observations per animal per day represented one day. Sometimes an identified animal was not found by the observers. For the autumn and the winter-spring period respectively 19 and 16 identified animals were used to study which identified individuals were seen together direct or indirect in the same group within a fixed time period. These identified animals were the same animals as were used for the focal sampling method. During autumn and winter-spring one observation per animal per day represented 1 day. Two groups were considered distinct if the distance between them was more than 50 meters. The distance of 50 meters was chosen for practical reasons.

Table 1: Detailed description of the types of contacts that have been observed in this study.

Type of contact	Definition
Nose-to-nose contact	Two animals have a nose-to-nose contact
Genital sniffing*	An animal smells at the bum or genitals of another animal, often this is associated with coaxing
Herding*	A bull that herds a cow/young cow. Often this is associated with genital sniffing or licking
Touching*	An animal touches (not with its nose) another animal anywhere except the nose or the genitals
Sniffing*	An animal touches another animal with its nose (other than genital sniffing and nose-to-nose contact)
Copulation attempt*	Copulation attempt
Horn fighting	Two animals fight with their head and/or horns
Licking*	An animal licks another animal, with a distinction made between licking the nose, the genitals or the body
Pushing*	An animal pushes another animal with its body
Bumping*	An animal bumps into another animal which can be a head-head contact or a head-body contact
Grazing*	An animal grazes another animal with its head or body
Suckling*	A calf or a young animal suckles its mother

* Note that for example "herding" could also mean that the observed cow was herded by a bull. The contact "herding" was then noted for the observed cow. Or "suckling" could also mean that a cow was suckled by her calf. The contact "suckling" was then noted for the observed cow. The same was true for the other types of contact.

It was assumed that all types of contacts gave a certain chance for BHV1 transmission. The contacts were observed between Heck cattle that live in a Dutch nature reserve 'the Oostvaardersplassen'. The observation period was divided into three study periods namely: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002).

Data analysis of the observed contact structure

Homogeneous population

The mean number of contactees per 20 minutes, the mean number of contacts per contactee per 20 minutes and the percentages of time spent on all types of contacts were calculated. For the autumn and the winter-spring period also a minimum number of contactees was calculated by looking at all 20 minutes of observation together of each identified animal. For each identified individual the highest number of different non-identified animals with whom contact was made (during one observation of 20 minutes out of all the observations of 20 minutes) was taken and added to all the different identified animals with whom contact

was made during all 20 minutes of observation. As was explained earlier it was tried to daily observe an identified animal for 20 minutes during autumn and winter-spring.
The calculation of the number of contactees when looking at all 20 minutes of observation together for an identified animal (e.g. animal *A*) was done as followed: suppose that animal *A* contacted animal *B* (cow) and a non-identified animal (cow) on day 1 (i.e. first observation of 20 minutes) and four non-identified animals (two cows and two bulls) on day 2 (i.e. second observation of 20 minutes). The non-identified cows on day 1 and day 2 may or may not be identical, so summarising these two days, animal *A* would have had at least three contactees of the animal type cow (animal *B* on day 1 and two non-identified cows on day 2) and two contactees of the animal type bull (two non-identified bulls on day 2), making a minimum of five contactees in total. The number of contactees of each identified animal looking at all 20 minutes of observation together of that animal was calculated similarly.

Heterogeneous population at the animal type level
We were also interested in how the number of observed contactees was distributed over the five animal types as were mentioned already earlier. For that purpose we calculated the mean number of contactees per 20 minutes for each combination of animal types, thus for animal type bull with animal type bull and for animal type bull with animal type cow and so on. This resulted in a five by five contact matrix.

Heterogeneous population at the individual level
To study the contact structure at individual level we used the observations of how many times and with whom each identified animal was seen together direct or indirect in the same group during a certain time period. The fraction of the number of days an identified animal was seen together with another identified animal in the same group (i.e. the association index (*ai*) (Martin and Bateson, 1993)) was calculated as followed:

$$ai = \frac{N_{AB}}{N_A + N_B + N_{AB}} \qquad (1)$$

where N_{AB} represents the number of days animal *A* was seen together in the same group with animal *B*, N_A represents the number of days animal *A* was seen, but not together with animal *B* and N_B represents the number of days animal *B* was seen, but not together with animal *A*. As was mentioned earlier, two groups were considered distinct if the distance between them was more than 50 meters. For the autumn and winter-spring period *ai* was calculated on the basis of one observation per animal per day, representing one day. For the summer period *ai* was calculated on the basis of two or three observations per animal per day, also

representing one day. Single linkage cluster analysis, using the above association index and two threshold values, was performed on these data with GenStat (Payne, 2000). Single linkage, alternatively called closest neighbour clustering, defines the distance between two clusters as the smallest distance between any two samples in those clusters. In this way two individuals that shared a neighbour but were not neighbours themselves, were also clustered into the same group.

The two threshold values were based on two durations of BHV1 excretion. The longest duration is when a primary infectious individual excretes virus that lasts about fourteen days (i.e. long infectious period) (Wentink et al., 1993). A shorter duration of infectiousness arises when a latently infected individual excretes virus again, that lasts for about five days (i.e. short infectious period) (Bosch, 1997). These two infectious periods were chosen because we were interested in whether groups or individuals were isolated from other groups or individuals during one of these infectious periods. This would give us insight in to what extent the disease could spread within the population depending on whether the infection started in a primary infectious individual or in a latently infected individual. The first threshold value was 0.07, which meant that two identified animals were seen at least once in the same group within a period of fourteen days. The second threshold value was 0.2, which meant that two identified animals were seen at least once in the same group within a period of five days. The fraction of days that animals were seen together from all the days that they were seen at all was thus compared to these two above mentioned thresholds. Note it was not taken into account that when animal *A* was seen together in the same group with animals *B* and *C* within the short infectious period, the infection could have continued through animals *B* and *C* and thus increasing the infectious period.

Calculation of the potential for BHV1 transmission

The observed contact structure compared to random mixing at the population level

The contact structure of all observed individuals was given as the probability $p(k)$ (for $k = 0,1,2,\ldots,\infty$) that the number of contactees is k. From this distribution of the observed number of contactees, the reproduction ratio (R) was calculated. As a comparison the reproduction ratio under random mixing (Poisson distribution) was also calculated. The hypothetical effect on BHV1 transmission was then studied by comparing these two reproduction ratios. This comparison was done because it was not possible to estimate the absolute value of R as the probability (Q) of BHV1 transmission given that two individuals are each other contactee was not known. By assuming that Q had the same value in the Heck cattle population as in the random mixing population this parameter disappeared by dividing the reproduction ratio for the observed contact structure by the reproduction for

the random mixing contact structure. In order to make the comparison with random mixing we assumed that the average number of contactees was the same in both situations.

Derivation of the reproduction ratio (R)

To derive *R* we used a general network model that describes the distribution of contactees for a large population (Diekmann et al., 1998). A crucial assumption of this model is that each contact is a random sample of the possible contacts in that population. The contact structure is simplified in this general network model in that it ignores higher order contacts than between direct neighbours such as triangles, loops or other clusters.

The probability (P_i) of having exactly *i* newly infected individuals from *k*-1 susceptible contactees follows for the general network model a binomial distribution. The number of contacts of each typical infectious individual for which P_i yields is one contact less (*k*-1) than the number of contacts of a random individual because one contact belongs to the individual through whom the typical infectious individual itself became infected. The probability P_i was calculated as followed:

$$P_i = \binom{k-1}{i} Q^i (1-Q)^{k-1-i} \quad (i = 0,1,2,\ldots,k-1) \qquad (2)$$

Any typical individual that has exactly *k* number of contactees will after infection have *k*-1 susceptible individuals left to infect. When all individuals in the population have exactly *k* contactees then the reproduction ratio *R* would be:

$$R = (k\text{-}1)Q \qquad (3)$$

Note the threshold value for the number of contactees *k* is 2. Since the number of contactees *k* of the individuals as observed, is a random number rather than a fixed quantity, we now account for this heterogeneity and generalise (3) by taking its expected value for the appropriate distribution of *k*. This yields:

$$R = Q\sum_{k=1}^{\infty}(k-1)\nu_k \qquad (4)$$

where ν_k is the distribution of the number of contactees *k* for a typical infectious individual. Note this is a different distribution than that of the number of contactees of a random individual in the population. More weight needs to be given to those individuals that have a large number of contactees because they have a higher probability of being contacted themselves. The probabilities ν_k follow from dividing $k\mu_k$ by the mean number of contactees ($E_\mu(k)$): $\nu_k = \frac{k\mu_k}{E_\mu(k)}$ (5)

Rewriting (4) using (5) gives the general formula for *R* for a population with any distribution of contactees:

$$R_{Heckcattle} = Q\left(\frac{Var_{\mu_k}\underline{k}}{E_{\mu_k}(\underline{k})} + E_{\mu_k}(\underline{k}) - 1\right) \qquad (6)$$

Var $\underline{k}$ is the variance of the number of contactees. This result shows that *R* increases with Var $\underline{k} > E(\underline{k})$ given the above assumptions. The numbers of contactees that individuals have in a random mixing herd is Poisson distributed. In the special case of the Poisson distribution Var $\underline{k} = E(\underline{k})$ and (6) simplifies to:

$$R_{Poisson} = QE_{\mu_k}(\underline{k}) \qquad (7)$$

If $R > 1$, major and minor outbreaks of BHV1 may occur in the population and if $R < 1$ only minor outbreaks of BHV1 may occur in the population. To get $R > 1$ the mean number of contactees should be higher than one under random mixing assumptions (equation 6). To get $R > 1$ when $Var\ \underline{k} = 0$, the number of contactees *k* should be higher than two (equation 2).

Homogeneous population

For each study period *R* was calculated, as was described above, using the number of contactees of all observed animals per 20 minutes. For the above situations *R* was calculated for the observed contact structure and for a random mixing contact structure assuming that the average number of contactees was the same in both situations.

After the calculation of the two reproduction ratios according to equations 6 and 7, an expression was derived for the ratio (*B*) of the *R* of BHV1 in the Heck cattle population to the *R* of BHV1 in a random mixing population. This ratio *B* was determined as followed:

$$B = \frac{Var_{\mu_k}\underline{k}}{\left(E_{\mu_k}(\underline{k})\right)^2} + 1 - \frac{1}{E_{\mu_k}(\underline{k})} \qquad (8)$$

If *B* turned out to be significantly different from one, then it would be concluded that the contact structure of the Heck cattle population did differ from random mixing. If *B* was less than one then the observed contact structure was more regular compared to random mixing. If *B* was higher than one then the observed contact structure was more clustered

compared to random mixing. The confidence intervals for *B* were estimated by randomly drawing 100,000 times a possible outcome for *B* from the probability distributions of the number of contactees with Mathematica® (Wolfram, 1999). The two-sided 95% confidence interval was derived in that way.

Heterogeneous population at the animal type level

Validity check of our contact data

The symmetry of a contact implies that the total number of contactees of *A* (focal animal type) with *B* (contactee animal type) was the same as the total number of contactees of *B* with *A*. In this study a contact between two animals was counted only once for the focal animal. To check the validity of the data the mean number of contactees of *A* with *B* and of *B* with *A* were calculated. Then the mean number of contactees of *B* with *A* was corrected for the difference between the total number of animals of each animal type in the general population, denoted as the correction factor. The mean number of contactees of *B* with *A* was then multiplied with this correction factor. This was done for all possible combinations of animal types. After correction the mean number of contacts of *A* with *B* should be the same as the mean number of contacts of *B* with *A*. Therefore it was tested whether the difference between the corrected means differed from zero. To that end the distribution of the difference based on the observed (marginal) distributions of contacts for *A* and *B* separately was calculated. In the calculations the correction for the differences in the number of *A* and *B* animals was taken into account. With this distribution of the difference the probability was calculated that the observed difference or larger was found. If this probability was higher than 0.05 then the difference between the corrected means differed from zero.

Reproduction ratio and ratio B

We were interested in whether animal types had randomly contact with each other. When contacts between animal types were not at random, we should take this heterogeneity at animal type level into account in the calculation of the reproduction ratio. The mixing structure at animal type level was studied by comparing the observed number of contactees for animal type *A* with animal type *B* with the expected number of contactees, based on separable mixing, for animal type *A* with animal type *B*. Separable mixing is the condition that the number of contactees an animal type of a focal animal has, is independent of the animal type of the contactee (see also Diekmann and Heesterbeek, 2000). The number of contactees focal animals had were entered in a two-way contingency table with focal animal type (i.e. the animal type of the individual that was observed) and contactee animal type (i.e. the animal type of the individual with whom contact was made) as dimensions. The test

used was the Pearson chi-square test and the exact p-value was approximated using Monte Carlo techniques with StatXact (Metha and Patel, 1998). For each cell in the contingency table the standardized cell residual was compared with the chi-square contribution per cell. A cell residual is considered to be a large deviate, when it is higher than the square root of the critical value of $\chi^2_{.05;d.f.}$ divided by the total number of cells (Bishop et al., 1975).
The reproduction ratio was also calculated by taking the heterogeneity at animal type level into account. A five by five next generation matrix was generated for the mean numbers of contactees per 20 minutes for each combination of animal types. *R* is then equal to the dominant eigenvalue of this five by five next generation matrix (Diekmann et al., 1990). *R* was calculated for the observed contact structure at animal type level and for a random mixing contact structure at animal type level. These two reproduction ratios were then used to calculate the ratio *B*. Both the reproduction ratio and the ratio *B* were compared with the reproduction ratio and the ratio *B* for the homogeneous population.

Results

Observation of the contact structure

In total a number of 42, 583 and 872 scan samples and 277, 306 and 456 focal samples were taken during summer, autumn and winter-spring, respectively. The large difference in the number of scan samples between the summer period and the other two study periods was due to the much larger and therefore fewer groups that were observed during summer.

Homogeneous population

Figure 1 illustrates the probability distributions of the number of contactees of all observed animals for each study period. Figure 1 shows that during each study period the highest probability was having zero contactees. During the winter-spring period the probability at having zero contactees was highest. During that period about 90% of the observed animals had zero contactees in 20 minutes of observation. The number of contactees ranged from 0 to 12, from 0 to 6 and from 0 to 3 during summer, autumn and winter-spring, respectively.
Table 2 gives the mean number of contactees per 20 minutes and the mean number of contacts per contactee per 20 minutes for each study period. For the autumn and winter-spring period Table 2 also gives the mean number of contactees when looking at all 20 minutes of observation together. Both the mean number of contactees and the mean number of contacts per contactee were highest during summer and lowest during winter-spring. The number of contacts per contactee ranged from 0 to 23, from 0 to 15 and from 0

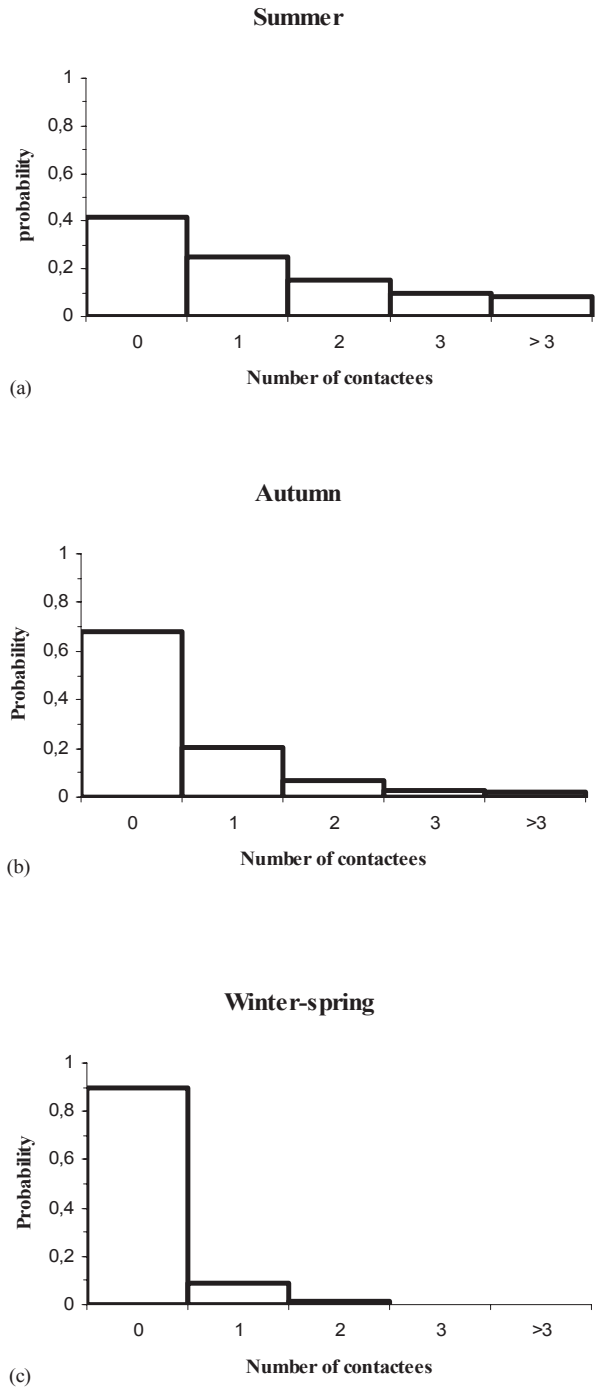

Fig.1. The probability distributions of the number of contactees of all focal animals per 20 minutes for each study period. The observation period was divided into three study periods namely: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). Contacts were observed between Heck cattle that live in a Dutch nature reserve 'the Oostvaardersplassen'. It was assumed that all types of contacts gave a certain chance for BHV1 transmission.

to 9 during summer, autumn and winter-spring, respectively.
The mean numbers of contactees for a longer observation time than 20 minutes were both higher during autumn and winter-spring than the mean numbers of contactees per 20 minutes. This was expected because the highest number of different non-identified animals with whom contact was made, was taken and added to all the different identified animals with whom contact was made.
The percentage of time spent on having contact with an animal with a certain probability of BHV1 transmission during summer, autumn and winter-spring was 5.3%, 2% and 1%, respectively.

Table 2: Mean number of contactees per 20 minutes (S.E.), mean number of contactees when looking at all 20 minutes of observation together (S.E.) and the mean number of contacts per contactee per 20 minutes (S.E.) for each study period.

Study period	Mean number of contactees per 20 minutes	Mean number of contactees for all 20 minutes of observation	Mean number of contacts per contactees per 20 minutes
Summer	1.30 (0.10)	N.A. *	2.69 (0.23)
Autumn	0.53 (0.06)	5.21 (0.75)	1.03 (0.13)
Winter-spring	0.12 (0.02)	2.13 (0.39)	0.20 (0.04)

* Not Available

The observation period was divided into three study periods namely: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). Contacts were observed between Heck cattle that live in a Dutch nature reserve 'the Oostvaardersplassen'. It was assumed that all types of contacts gave a certain chance for BHV1 transmission.

Heterogeneous population at the animal type level

During summer bulls had the highest number of contactees and most contactees were observed between bulls and cows. Cows had the lowest number of contactees, whereas most contactees were of the animal type cow. During autumn young bulls had the highest number of contactees and most contactees were observed between young bulls. Bulls had the lowest number of contactees, whereas most contactees were of the animal type bull. During winter-spring cows had the highest number of contactees and most contactees were observed between young bulls and cows. Young cows had the lowest number of contactees. Most contactees were of the animal type cow.

Heterogeneous population at individual level

We studied the heterogeneity at individual level by observing the number of times an identified animal was seen together in the same group with another identified animal within a fixed time period. The analysis was done for 49, 19 and 16 identified individuals during summer, autumn and winter-spring, respectively.

During summer one bull group (i.e. two bulls) was separated from a rest group (i.e. 47 individuals) for the first threshold value of one sighting per 14 days and three bull groups were separated from a rest group for the second threshold value of one sighting per five days. During autumn one young bull and a cow were both separated from a rest group (i.e. 17 individuals) for the first threshold value and twelve solitary animals, two cows, two calves and one young cow and two calves were separated for the second threshold value.

During winter-spring one bull group (three bulls and a male calf) and one solely bull were separated from a rest group (i.e. 11 individuals) for the first threshold value and fourteen solitary animals and two bulls were separated for the second threshold value.

Summarizing, during summer most individuals were seen together in the same group except for certain bull groups. During autumn and winter-spring most individuals were seen together in the same group for the long infectious period of fourteen days. However, the group was fallen apart in solitary animals and small groups for the short infectious period of five days.

Calculation of the potential for BHV1 transmission

Homogeneous population

First two reproduction ratios were calculated from the distribution of the number of contactees of all observed individuals using equations 6 and 7. Ratio *B* (equation 8) was then calculated by dividing the *R* of BHV1 for the observed contact structure (equation 6) by the *R* of BHV1 for a random mixing contact structure (equation 7). Table 3 gives the estimates for the ratio *B*. For each study period the point estimates for *B* were above one, which meant more variation in the number of contactees than under random mixing. The observed contract structure did significantly differ from random mixing as all confidence intervals did not include the value of *B* is one.

Heterogeneous population at the animal type level

For most animal types during summer and autumn the total number of contactees of *A* with *B* was not the same as the total number of contactees of *B* with *A*. For the winter-spring period only the total number of contactees of cows with calves and the total number of contactees of calves with cows was not the same. Too few contacts for the other combinations of animal

types were observed during this period to find significant differences.

Table 3 : The estimates for the ratio *B* and its 95% two-sided confidence interval calculated from the distribution of the number of contactees per 20 minutes for each study period.

Study period	Ratio B	95% two-sided confidence interval
Summer	1.96	[1.52 – 2.48]
Autumn	2.64	[1.74 – 2.75]
Winter-spring	2.74	[1.01 – 4.59]

The observation period was divided into three study periods namely: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). Contacts were observed between Heck cattle that live in the Dutch nature reserve 'the Oostvaardersplassen'. It was assumed that all types of contacts gave a certain chance for BHV1 transmission.

Figure 2 shows the presence or absence of observed contacts between animal types. Different degrees for the mean number of contactees were indicated by the thickness or absence of the arrows. Sometimes a small number of contactees was observed for animal type *A* with animal type *B* whereas no contactees were observed for animal type *B* with animal type *A*, which could be due to chance. This was for example the case during summer between bulls and calves as could also be seen in Figure 2. In other cases as for example between cows and bulls during summer and autumn, there were high differences in the mean number of contactees between the animal types (see Figure 2). This could be due to the fact that the distribution of the number of contactees was more skewed for one of the animal types with the possibility that animals with many contacts were not equally represented in both animal types (compare Morris, 1993). We continued however working with the observed frequency distributions for the number of contactees as it was not possible to detect from our data the real total number of contactees of *A* with *B* and thus also of *B* with *A*.

The analysis of the contingency tables showed that the number of contactees an animal type of a focal animal had, was not independent from the animal type of the contactee for summer and autumn ($P << 0.01$) but was independent for winter-spring ($P = 0.08$). During summer and autumn there was thus no separable mixing at the animal type level. During the study period winter-spring too few contacts were observed to find a difference.

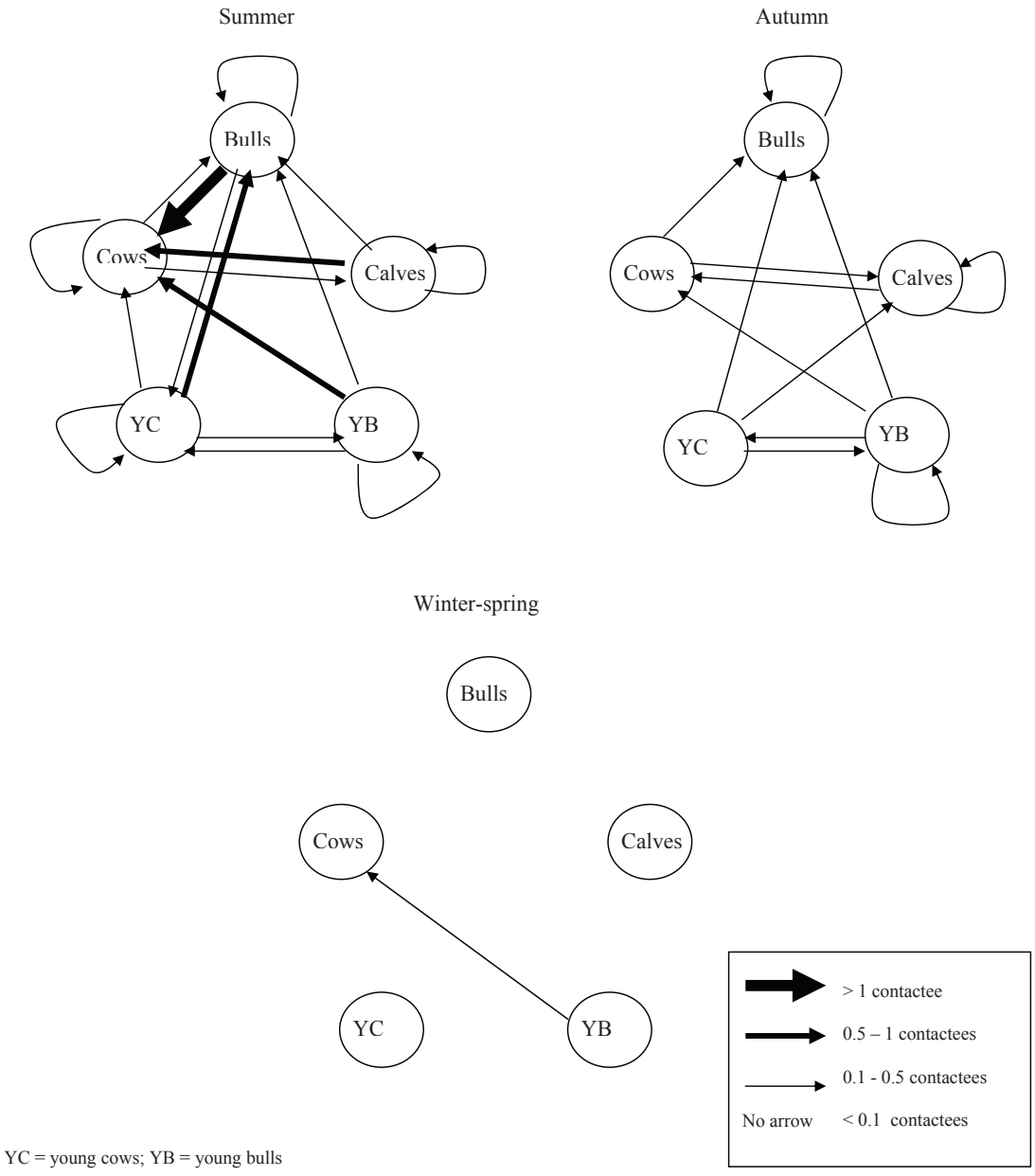

Fig.2. The presence or absence of contacts – measured by the average number of contactees per 20 minutes – observed between animal types for each study period. The thickness or absence of the arrows indicates the range of the observed average number of contactees between the animal types. The observation period was divided into three study periods namely: summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). Contacts were observed between Heck cattle that live in a Dutch nature reserve 'the Oostvaardersplassen'. It was assumed that all types of contacts gave a certain chance for BHV1 transmission. (Additional information: during summer the total number of animals per animal type in the eastern population was assumed to be 126 cows, 85 bulls, 22 YC, 26 YB and 56 calves and during autumn and winter-spring 119 cows, 82 bulls, 22 YC, 9 YB and 100 calves).

	C	B	YC	YB	Ca
	S A W	*S A W*	*S A W*	*S A W*	*S A W*
C	↓ ↓ ↓	↑ ↑ ↑	↓ ↓ *	↑ ↓ *	↑ ↑ ↑
B	↑ – –	↓ ↑ ↑	↑ ↑ *	↓ ↓ *	↓ ↓ ↓
YC	↓ ↓ *	↑ ↑ *	↓ ↓ *	↑ ↑ *	↓ ↓ *
YB	↓ – *	↓ ↓ *	↑ ↑ *	↑ ↑ *	↓ ↓ *
Ca	↑ ↑ ↑	↓ ↓ ↓	↓ – *	↓ ↓ *	↑ ↑ ↑

↑ = the standardized cell residual is more than the chi-square contribution per cell

↓ = the standardized cell residual is less than the chi-square contribution per cell

- = the standardized cell residual is within the range of the chi-square contribution per cell

* = no value available for the winter-spring period, for statistical reasons cows and young cows have been put into one female class and bulls and young bulls into one male class

(C = cow; B = bull; YC = young cow; YB = young bull; Ca = calf, S = summer, A = autumn, W = winter-spring)

Fig.3. Differences between the observed and expected, based on separable mixing, number of contactees between animal types. The focal animal type is given by each row *i* and the contactee animal type is given by each column *j*. The first, second and third symbol for each state (*i*,*j*) are related to the respective study periods summer (July 2001 to September 2001), autumn (October 2001 to November 2001) and winter-spring (February 2002 to April 2002). Contacts were observed between Heck cattle that live in a Dutch nature reserve 'the Oostvaardersplassen'. It was assumed that all types of contacts gave a certain chance for BHV1 transmission.

Figure 3 gives a more detailed description of the differences between the observed and expected, based on separable mixing, number of contactees between the animal types. For example during summer cows and young cows had more contactees of the types bull and young bull and bulls had more contactees of the types cow and young cow than expected. During autumn bulls and young bulls had more contactees of the type young cow and of their own type than expected and young cows had more contactees of the type bull and young bull than expected. During each study period calves had more contactees of the types calf and cow than expected and cows had more contactees of the type calf and less of their own type.

Because of the observed heterogeneity in the number of contactees between animal types, the contact structure at animal type level was taken into account in the calculation of the reproduction ratio and ratio *B* as this could influence the outcome of these parameters. The reproduction ratios for the observed contact structure, when taking heterogeneity at animal type level into account, were higher than the reproduction ratios for the homogeneous population. The values for ratio *B* were 2.3, 4.0 and 4.4 for the summer, autumn and winter-spring period, respectively and were also higher than the values for ratio *B* for the homogeneous population (see Table 3).

Discussion

In this study we quantified the contact structure of part of a Heck cattle population in a Dutch nature reserve and its hypothetical effect on BHV1 transmission.

First of all we wanted to know with how many different animals an animal had contact. Therefore we had made a protocol for observing the number of contactees per 20 minutes. These 20 minutes were not taken randomly over 24 hours but these 20 minutes were distributed over a period from 9 a.m. till 4 p.m. We also estimated the number of contactees when looking at all 20 minutes of observation together. In this way we were able to estimate the number of contactees for a longer observation period, which corresponded more to the duration of BHV1 excretion. Some limitations of the above mentioned protocol for observing the number of contactees are discussed here. No data were gathered outside the observation period whether cattle might of course also be active outside that period. It was however not known whether an observation of 20 minutes in the period between 4 p.m. and 9 a.m. would be different from an observation of 20 minutes during daylight. It could thus not be said if the observed number of contactees was underestimated or overestimated. However, when looking at all 20 minutes of observation together, the number of contactees could only be the same or higher when we would also have taken observations between 4 p.m. and 9 a.m. into account. We chose for practical reasons to estimate the number of contactees during daylight. Further the number of identified individuals that were observed during autumn and winter-spring, which was necessary to be able to observe these animals daily, was limited. Extrapolating the results to the whole eastern population of Heck cattle should therefore be done with care.

Second, we were interested in the hypothetical effect of the observed contact structure on the potential for BHV1 transmission and on the persistence of BHV1 in the Heck cattle population. One of the effects was a seasonal effect in the number of observed contacts. The number of contactees and the number of contacts per contactee were highest during summer and lowest during winter-spring. This suggests that transmission would be favoured most during summer. Further the mean number of contactees per 20 minutes during summer was already sufficient to get R above 1 under random mixing assumptions. During autumn and winter-spring the mean numbers of contactees when looking at all 20 minutes of observation together were also above one. The reproduction ratios for the observed contact structure were higher for the heterogeneous population at animal type level than for the homogeneous population. Heterogeneity at animal type level thus also influenced the potential for BHV1 transmission. From the observations at individual level we established that the whole population did not mix freely. This also influenced the potential

for BHV1 transmission as when social groupings live isolated from each other during time periods longer than the period of disease outbreaks, the epidemic might be limited to the group in which the infection is started.

The hypothetical effect of the observed contact structure would probably be limited on the persistence of BHV1 because it was already shown that even when groups were small BHV1 is able to survive for a long period in cattle populations without the introduction of the virus from outside the population (De Koeijer, 2003; Mollema et al., 2005). The aforementioned authors showed that for realistic parameter values (*R* above 1), BHV1 may survive for more than hundred years in a demographically stable cattle population of ten animals.

Besides sufficient contact, other conditions must be fulfilled for BHV1 to persist, such as the birth of new susceptible animals and reactivation of the virus in a latently infected individual. Each year about sixty susceptible calves were born in the Heck cattle population of about 320 animals (J. Griekspoor, pers. com.). Most animals were born within the months February to April, which is the winter-spring period in this study. Note calves might be protected against BHV1-infection by maternal antibodies for one till six months (Kahrs, 1977) after which they become susceptible again. If we assume that maternal antibodies are lost after six months and that most calves were born in March than calves will be susceptible again in September, which is the end of the summer period. If calves do not have maternal antibodies then calves are already susceptible at birth and most births take place in the winter-spring period.

Reactivation of BHV1 has been observed in latently infected domestic cattle due to stress (i.e. increase of corticosteroids), for example in the form of transport or parturition (Wentink et al., 1993). In a field study with red deer (*Cervus elaphus*) it was shown that fecal glucocorticoid excretion varied seasonally with a peak during December and January (Huber et al., 2003). Huber et al. (2003) suggested that high winter glucocorticoid levels might act via catabolic function during adaptation of deer to the cold winter months when resources are limited. In experiments with mice De Groot et al. (1999) showed that corticosterone levels were elevated immediately by social defeat stress. A single social defeat was applied at 3 or 6 days after inoculation with pseudorabies virus, a herpes virus. Given the above examples it was suggested that reactivation of BHV1 in Heck cattle might be due to starvation, harsh weather conditions, fighting, parturition or a combination of these stress conditions. Stress might occur in each of the study periods. Virus could thus at some moment of time during each of the study periods reactivate in latently infected animals due to one of the above stress conditions and be transmitted to susceptible animals.

The observed contact structure was used in this study to calculate the hypothetical effect of the contact structure on BHV1 transmission and to give us more insight in the transmission of BHV1 in the feral cattle population. However, the observed contact structure could also

be used to suggest: i) appropriate ways for taking blood samples in the cattle population to show with sufficient certainty that the population is free of other cattle diseases with the same short infectious period as BHV1 and similar transmission routes (e.g. Foot and Mouth Disease); or ii) an appropriate vaccination strategy for eradication of BHV1 in the Heck cattle population.
For an appropriate way of blood sampling and vaccinating animals it is important to determine the animal types with large numbers of contactees and the animal types with whom most contacts were made. For example in the summer period vaccinating cows and sexually active bulls would be an appropriate vaccination strategy. Modelling of BHV1 transmission in a partly vaccinated Heck cattle population could then give us insight into whether this vaccination strategy instead of vaccinating the whole Heck cattle population could be used to eradicate BHV1.

Conclusion

The number of contactees was highest during summer and lowest during winter-spring. The contact structure of the homogeneous population did differ significantly from a random mixing contact structure. The variation in the number of contactees was higher than under random mixing.
Bulls, young bulls and cows had the highest number of contactees during respectively summer, autumn and winter-spring. From the analysis of the contingency tables it was clear that contacts between animal types did not occur at random during summer and autumn. For example, during summer more contacts occurred between bulls and cows than expected. This heterogeneity at animal type level was taken into account in the calculation for *R*, which resulted for the observed contact structure in higher estimates for *R* than for the homogenous population.
When looking at heterogeneity at individual level it was found that during summer almost all individuals were observed together direct or indirect in the same group except for certain bull groups for both infectious periods of BHV1. During autumn and winter-spring almost all individuals were seen together in the same group when considering a long contact period but the groups were fallen apart in small groups and solitary individuals for a shorter contact period.
It could be concluded that based on the observed contact structure transmission would be favoured most during summer.

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References

Bishop, Y.M.M., Fienberg, S.E., Holland, P.W. (eds.), 1975. Formal goodness of fit: summary statistics and model selection. In: Discrete multivariate analysis: theory and practice. The MIT Press, Cambridge, pp. 136-137.

Bosch, J.C., 1997. Bovine Herpesvirus 1 marker vaccines: tools for eradication? Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 122.

Diekmann, O., Heesterbeek, J.A.P., Metz, J.A.J., 1990. On the definition and computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J. Math. Biol. 28, 365-382.

Diekmann, O., De Jong, M.C.M., Metz, J.A.J., 1998. A deterministic epidemic model taking account of repeated contacts between the same individuals. J. Appl. Prob. 35, 448-462.

Diekmann, O., Heesterbeek, J.A.P., 2000. What is contact? In: S. Levin (ed.), Mathematical epidemiology of infectious diseases. John Wiley & Son, LTD, Chichester, pp. 153-169.

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpesviruses. Part I: bovine herpesvirus 1. Vet. Bull. 47, 317-343.

De Groot, J., Van Milligen, F.J., Moonen-Leusen, B.W.M., Thomas, G., Koolhaas, J.M., 1999. A single social defeat transiently suppresses the anti-viral immune response in mice. J. Neuroimmunol. 95, 143-151.

Huber, S., Palme, R., Arnold, W., 2003. Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). General Comparative Endocrinol. 130, 48-54.

Kahrs, R.F., 1977. Infectious bovine rhinotracheitis: A review and update. JAVMA 171, 1055-1064.

De Koeijer, A.A., 2003. Infectious diseases among animals: combining models and data. Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 152.

Lazo, A., 1994. Social segregation and the maintenance of social stability in a feral cattle population. Anim. Behav. 48, 1133-1141.

Mars, M.H., 2000. Vaccinology and Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, The Netherlands, pp 145.

Martin, P., Bateson, P. (eds.), 1993. Recording methods. In: Measuring behaviour: an introductory guide. University Press, Cambridge, pp. 84-100.

Metha, C., Patel, N. (eds.), 1998. StatXact 4 for windows. CYTEL Software Corporation, Cambridge.

Mollema, L., De Jong, M.C.M., Boven, M., 2005. Prolonged persistence of bovine herpesvirus in small cattle herds: a model based analysis. Epidem. Infect. 133, 137-148.

Morris, M., 1993. Telling tails explain the discrepancy in sexual partner reports. Nature 365, 437-440.

Payne, R. (ed.), 2000. The guide to GenStat. VSN International Ltd, Oxford.

Sinclair, A.R.E., 1977. Social behavior. In: Schaller, G.B. (ed.), The African Buffalo: a study of Resource Limitation of Populations. The University of Chicago Press, Chicago, pp.
101-155.

Vulink, JTh., 2001. Hungry Herds: management of temperate lowland wetlands by grazing. Ph.D. Thesis. University of Groningen, The Netherlands, pp 394.

Van Vuure, C. (ed.), 2005. Retracing the Aurochs: history, morphology and ecology of an extinct wild ox. Pensoft, Sofia-Moscow, pp 431.

Wentink, G.H., Van Oirschot, J.T., Verhoeff, J., 1993. Risk of infection with bovine herpesvirus 1 (BHV1): A review. The Veterinary Quarterly 15, 30-33.

Wolfram, S. (ed.), 1999. The Mathematica Book. University Press, Cambridge.


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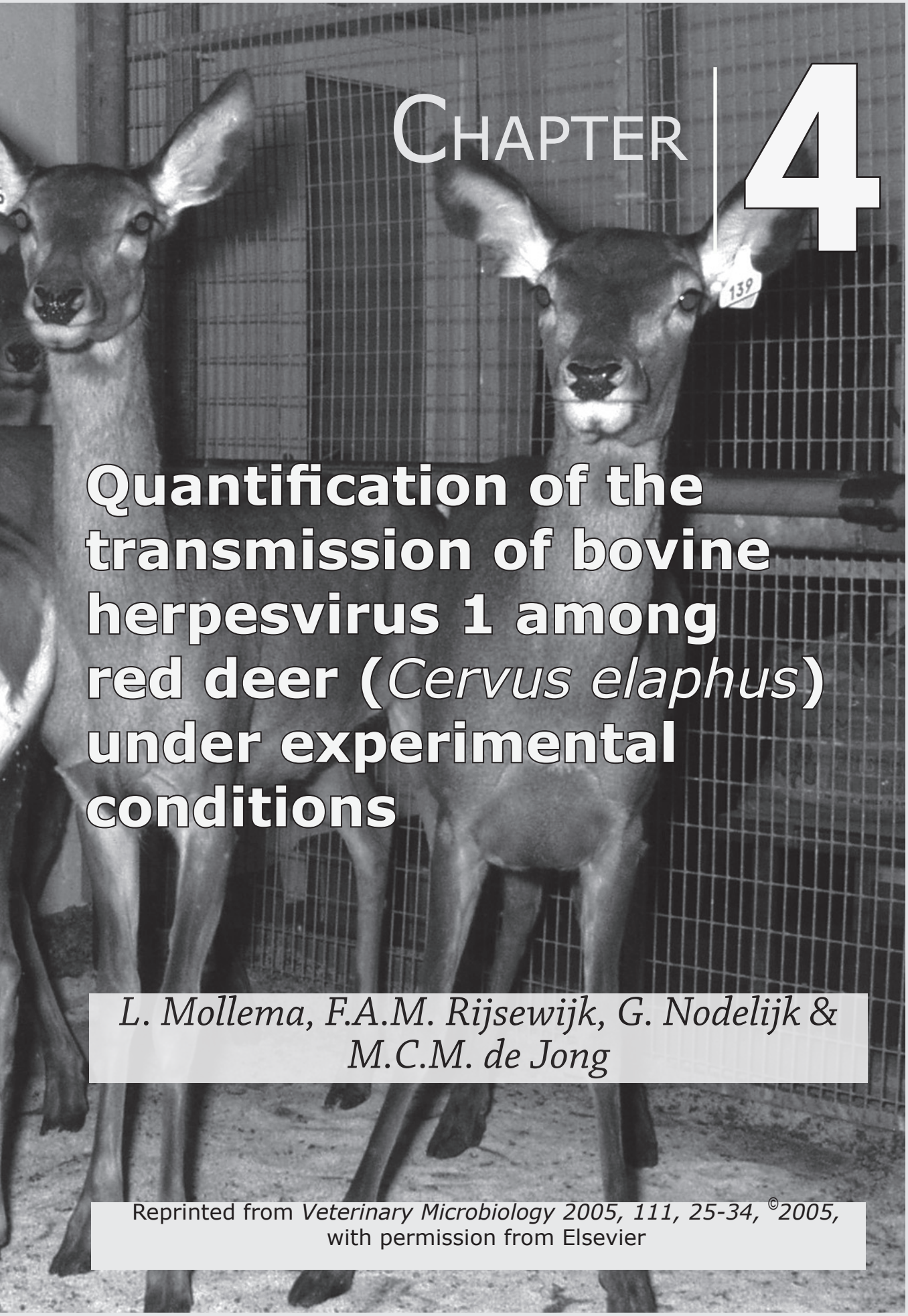

CHAPTER 4

Quantification of the transmission of bovine herpesvirus 1 among red deer (*Cervus elaphus*) under experimental conditions

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Summary

Bovine herpesvirus 1 (BHV1) is endemically present in a cattle population that lives in a nature reserve in the Netherlands. Red deer (*Cervus elaphus*), living in the same nature reserve, can come into contact with the BHV1-infected cattle and could then become infected with BHV1. For the eradication of BHV1 in cattle, it is, therefore, important to know whether red deer alone can play a role in the transmission of BHV1. For that reason, we quantified the transmission of BHV1 among farmed red deer under experimental conditions. Two groups of ten animals were formed. In each group, five of these animals were inoculated with BHV1 and the other five served as contact animals.

Three inoculated animals in each transmission experiment became infected and none of the contact animals became infected. The one-sided 95% confidence interval for R [0.0 – 0.94] showed that limited transmission might occur among red deer. Based on these results, we would expect only minor outbreaks of BHV1 to occur in red deer populations. We concluded that BHV1 will probably not survive longer than a few decades (several times the mean deer lifetime) in red deer populations. Consequently, it is not necessary for the eradication of BHV1 in cattle to eradicate BHV1 in red deer populations as well.

Introduction

Bovine herpesvirus 1 (BHV1) is an alphaherpesvirus that infects cattle causing infectious bovine rhinotracheitis (IBR) and genital infections (Gibbs and Rweyemamu, 1977). Several countries, including the Netherlands, have started programs aimed at eradication of BHV1 in domestic cattle. To that end, eradication of BHV1 in all core groups is necessary, whenever core groups hamper the eradication. Definitions for a core group exist mainly in the field of sexually transmitted diseases (Thomas and Tucker, 1996). The term 'core' was later associated (Anderson, 1982) with the reproduction ratio *R*, which is the average number of newly infected animals caused by a single typical infected animal (Diekmann et al., 1990). A core group in this study was defined as a group of any smallest combination of populations for which *R* is larger than 1. For BHV1, cattle form certainly a core group (Bosch, 1997; Hage, 1997; Mars, 2000).

To understand the transmission dynamics of an infectious agent that can infect two or more different animal species (e.g. for BHV1, there can be several animal species) it is relevant to distinguish between core and satellite groups. Any group not belonging to a core group, but which can become infected, is called a satellite group.

In the Netherlands a cattle population of about 600 individuals lives in a nature reserve and most (110 out of 124) of these cattle that were tested in the period 1996-2003, were seropositive for BHV1 using a gB blocking ELISA. This cattle population is, therefore, probably also a core group for BHV1. A red deer (*Cervus elaphus*) population of about 1000 individuals lives together with this cattle population. As red deer can be infected with BHV1 (Reid et al., 1986) and can come into contact with these BHV1-infected cattle, they can also become infected with BHV1. Therefore, the question arises whether red deer also form a core group for BHV1. This means that it is interesting to know whether BHV1 can survive among red deer without the interaction with BHV1-infected cattle. A necessary but not sufficient condition for the survival of an infectious agent in a population is that such an agent can cause a major outbreak [$R \geq 1$]. If e.g. $R < 1$ for BHV1 in a certain animal species population, BHV1 will probably go extinct in that population within a few decades. It takes at least several times the mean animal lifetime of the animals before the virus goes extinct in this population, because even when $R < 1$ some individuals of such a population can still become infected with BHV1. This is due to the property of BHV1 and other alphaherpesviruses, that once such a virus infects an individual, it remains in that individual for life. Under certain conditions, BHV1 can reactivate and the carrier hosts become infectious again (reviewed by Jones, 2003). BHV1 will, therefore, persist in a population until all carrier animals have died or have been taken out of that population.

To determine whether red deer form either a core or a satellite group for BHV1, it is important to estimate the *R* for BHV1 in red deer. Therefore, two five-to-five transmission experiments were performed in red deer.

Material and Methods

Two types of experiments

Before the two transmission experiments were performed an inoculation experiment was done to investigate: i) whether red deer inoculated with the BHV1 strain used in this study excreted virus and developed antibodies against BHV1 and ii) whether the BHV1 gB blocking ELISA developed by our institute (Kramps et al., 1994) was able to detect red deer antibodies against BHV1. For the inoculation experiment, two red deer were inoculated with BHV1 and two other red deer were inoculated with a red deer herpesvirus, cervid herpesvirus 1 (CeHV1), as a positive control.

Animals and housing

For the inoculation experiment, four 8-month-old farmed female red deer were used. The four animals were randomly assigned to two groups of two animals and the groups were housed separately. One group was intranasally inoculated with BHV1 and the other group was intranasally inoculated with CeHV1.

For the transmission experiments twenty 10-12-month-old farmed female red deer were used. Two identical transmission experiments were carried out one after the other. For each experiment ten animals were randomly assigned to two groups of five animals. One group was intranasally inoculated with BHV1 and the other group was kept as a contact group. The number of ten animals per experiment was chosen because when conducting two of such experiments, it can have satisfactory power to find a significant difference from $R \geq 1$ (Kroese and De Jong, 2001; Velthuis, 2002).

Blood from all animals was collected and tested in the BHV1 gB blocking ELISA (Kramps et al., 1994) before starting the experiments to confirm that all animals were seronegative to BHV1 and/or CeHV1.

The inoculation experiment was carried out in two isolation units (25 m^2) at the facilities of the Animal Sciences Group in Lelystad. The transmission experiments were carried out in another isolation unit (30 m^2) at the same facilities. At the start of both transmission experiments, five randomly assigned animals, denoted further as contact animals, were isolated from the other five animals, denoted further as inoculated animals, by placing them behind a wooden partition to prevent physical contact between the inoculated and contact

group for the first 24 hours after inoculation. The five animals that remained were intranasally inoculated with BHV1. Twenty-four hours after inoculation a door in the wooden partition was opened and the animals could mingle freely. The air conditioning was turned off from 11 a.m. till 5 p.m. at the day of inoculation. To prevent cross-infections by the researchers and/or animal caretakers, these persons applied strict rules for hygiene. Animal caretakers first took care of the contact animals and changed gloves each time before taking care of the next animal.

Viruses and cells

CeHV1 was grown on Mardin-Darby bovine kidney (MDBK) cells and was a kind gift of the Moredun Research Institute, Edinburgh. CeHV1 was inoculated at 10^7 50% tissue culture infective dose ($TCID_{50}$) per ml, 1 ml in each nostril. BHV1 (Lam strain) was grown on embryonic bovine tracheal cells (EBTr) and was isolated from an outbreak of bovine rhinotracheitis (IBR) in the Netherlands in 1972 (Kaashoek et al., 1996). BHV1 was inoculated using the same titre as for CeHV1.

Experimental designs

Two weeks before the start of each experiment, the animals were housed as mentioned above. These two weeks were used to allow the animals to accustom to their new environment, to the handlings and to see whether the animals could be kept together, which was the case in all three experiments.

The animals of the inoculation experiment were followed for five weeks after inoculation.

The inoculated animals of each transmission experiment contact exposed the contact animals for four weeks after inoculation. At the end of the experiments, all the animals were euthanised.

Sampling procedures

Clinical scores

For the inoculation experiment, the clinical scores (for nasal and ocular discharges, lesions in nose, appetite and abnormalities in behaviour) of all animals were recorded daily for two weeks (D_0-D_{14}). The clinical parameters nasal and ocular discharges and lesions in nose were scored 0 when not present, 1 when mild and 2 when severe. Appetite was scored 0 when the animals ate the daily amount of food, 1 when they ate but not the daily amount of food and 2 when the animals did not eat. Behaviour was scored as 0 when the animals did not react, 1 when they were quiet, 2 when they had average behaviour, 3 when they were lively and 4 when they had restless behaviour.

The rectal temperatures of all animals of the inoculation experiment were recorded at D_{-14} and daily for two weeks (D_0-D_{14}).
For the transmission experiments, the clinical scores, as were described above, of all animals were recorded daily for six weeks (D_{-14}-D_{28}). The rectal temperatures of the inoculated animals were recorded daily for two weeks (D_0-D_{14}) and of the contact animals daily for three weeks (D_0-D_{21}).

Nasal swabs
Plain synthetic swabs were used for virus isolation by rotating them several times in one nostril of an animal. For the inoculation experiment, nasal swabs of all four animals were taken daily for two weeks (D_0-D_{14}) and thereafter once a week at D_{21}, D_{28} and D_{35}.
For the two transmission experiments, nasal swabs of the inoculated animals were taken daily for two weeks (D_0-D_{14}) and thereafter, once a week at D_{21} and D_{28} and of the contact animals daily for four weeks (D_0-D_{28}).

Blood samples
All blood samples were taken from one of the jugular veins. For the inoculation experiment, blood samples of all four animals were taken at D_{-24} and D_{-14} and starting at D_0 once a week until the end of the experiment.
For the two transmission experiments, the first blood samples were taken at D_{-16} and D_{-19}, respectively, and thereafter, once a week, starting at D_0 until the end of the experiments

Handling of the animals during the transmission experiments

In all isolation units, a second wooden partition was placed. This partition was fixed at one side, while the other side could be moved. The animal caretakers managed such that five animals were present at each side of the other wooden partition, described earlier in the animals and housing section, which made it easier to carry out the handlings. When taking the nasal swabs and rectal temperatures, all five animals were kept together behind the moving partition by one animal caretaker, while the other person carried out the handlings. When taking the blood samples, each animal was placed separately behind the moving partition with its head rising above the partition. One person was keeping the animal behind the partition, the second person was keeping the head fixed by holding its ears and the third person was taking the blood sample. It was not necessary to immobilise the animals.

Diagnostic tests

Virus isolation

The nasal swabs were suspended into 2 ml of Eagles Minimal Essential Medium with Earle's salts (EMEM), supplemented with 2% of fetal bovine serum and 0.7% of an antibiotic stock containing: 70 IU of penicillin per ml, 78.8 μg of streptomycin per ml, 35 IU of nystatin per ml and 70 μg of kanamycin per ml. After centrifugation (5 min. at 1500 x g), the supernatant was taken and stored in duplicate at –70°C to determine later on the presence of BHV1 and CeHV1 and to determine the virus titre. At the end of each experiment, all samples were thawed quickly and tested in 96-well plates. All the samples taken in the inoculation experiment were tested in duplicate. Most of the samples taken in the first and the second transmission experiment were tested three times and in duplicate, respectively.

To each well, 100 μl of a suspension of Mardin-Darby bovine kidney (MDBK) cells (approximately 100,000 cells) in Eagles Minimal Essential Medium with Earle's salts supplemented with 10% fetal bovine serum and 0.7% of the antibiotic stock was added. The following day, samples of 50 μl of each dilution were added to the cells. After five or six days of incubation at 37°C and 5% CO_2, the cultures were examined for the development of cytopathic effect (cpe) typical for BHV1 and CeHV1 (Gibbs and Rweyemamu, 1977).

Virus titration

To determine virus titres, virus-positive samples were thawed quickly. Ten-fold serial dilutions were made in culture medium (EMEM, supplemented with 10% fetal bovine serum and 0.7% of the antibiotic stock). To each well 100 μl of a suspension of MDBK cells was added. The following day, samples of 50 μl of each dilution were added to the cells in one row of 6 wells. After an incubation period of five to six days at 37°C and 5% CO_2, the cultures were examined microscopically for cpe. Virus titres were calculated according to the method of Reed-Muench (Reed and Muench, 1938) and expressed as $^{10}\log TCID_{50}$ per ml of nasal fluid.

Serological examination

The presence of antibodies against BHV1 and CeHV1 in red deer was determined in serum by using the BHV1 gB blocking ELISA of Kramps et al. (1994).

An animal was considered seropositive to BHV1 or CeHV1 if a blocking percentage of ≥ 50% was measured in this BHV1 gB ELISA. The BHV1 gB ELISA was also used to detect antibodies against CeHV1 in red deer inoculated with CeHV1. In this ELISA, a monoclonal antibody against glycoprotein gB of BHV1 was used to be blocked by antibodies in test sera. Glycoprotein gB is one of the major antigens of both BHV1 and CeHV1. Ros and Belák (1999)

demonstrated that the predicted amino acid sequences of glycoproteins gB of both viruses show 94.8% identity. It was thus expected that antibodies against gB of CeHV1 would cross-react in the BHV1 gB ELISA used in this study (see also Lyaku et al., 1992; Nixon et al., 1988).

Data analyses of the transmission experiments

Final size analysis

To determine to what extent BHV1 can be transmitted among red deer the reproduction ratio *R* was calculated. A 'general epidemic model' (Bailey, 1975) also called a 'Susceptible-Infective-Removed' ('SIR') model (Dietz, 1982) was used to describe the probability distribution of the outcome of a transmission experiment (final size) in terms of *R*. In De Jong and Kimman (1994) an algorithm is described to calculate this probability distribution. The *R* was estimated by Maximum Likelihood Estimation (MLE), i.e. it was calculated which value of *R* gave the highest combined likelihood for the observed number of contact infections in the two transmission experiments. A one-sided statistical test about *R* was performed by calculating the exact *P*-value for all extreme values (Kroese and De Jong, 2001) and the one-sided 95% confidence interval for *R* was calculated.

For the above analysis of the data from the two transmission experiments it is essential to determine the status (*S* = susceptible, *I* = infectious or *C* = recovered) of each individual animal. At the start of each experiment, all animals were assumed to be susceptible, since all animals were tested negative in the BHV1 gB blocking ELISA. All animals were assumed to be equally susceptible and when infected equally infectious. Inoculated animals were considered to be infected if: i) virus could be detected in the nasal secretions; or ii) an animal became seropositive to BHV1 in the BHV1 gB ELISA. Therefore, transmission of BHV1 had occurred if virus was detected in the nasal secretions of one of the contact animals or if a contact animal became seropositive to BHV1 in the BHV1 gB ELISA.

Estimation of the infectious period

The estimation of the infectious period was based on the number of days of virus detection in each infected animal (inoculated and contact animals) of the two transmission experiments. Hence, estimation of the mean infectious period ($\hat{T}$) and corresponding confidence interval was straightforward.

Results

Clinical signs

Inoculation experiment

The two animals inoculated with BHV1 showed lively behaviour during the whole experiment. None of the animals showed some nasal discharge or other clinical signs. The temperatures of both animals were relatively high (40.3°C and 39.1°C, respectively) before D_0 of the experiment. The temperature of one animal (animal 125) also showed an increase at day three after inoculation, coincidental with the detection of virus (Fig. 1A).

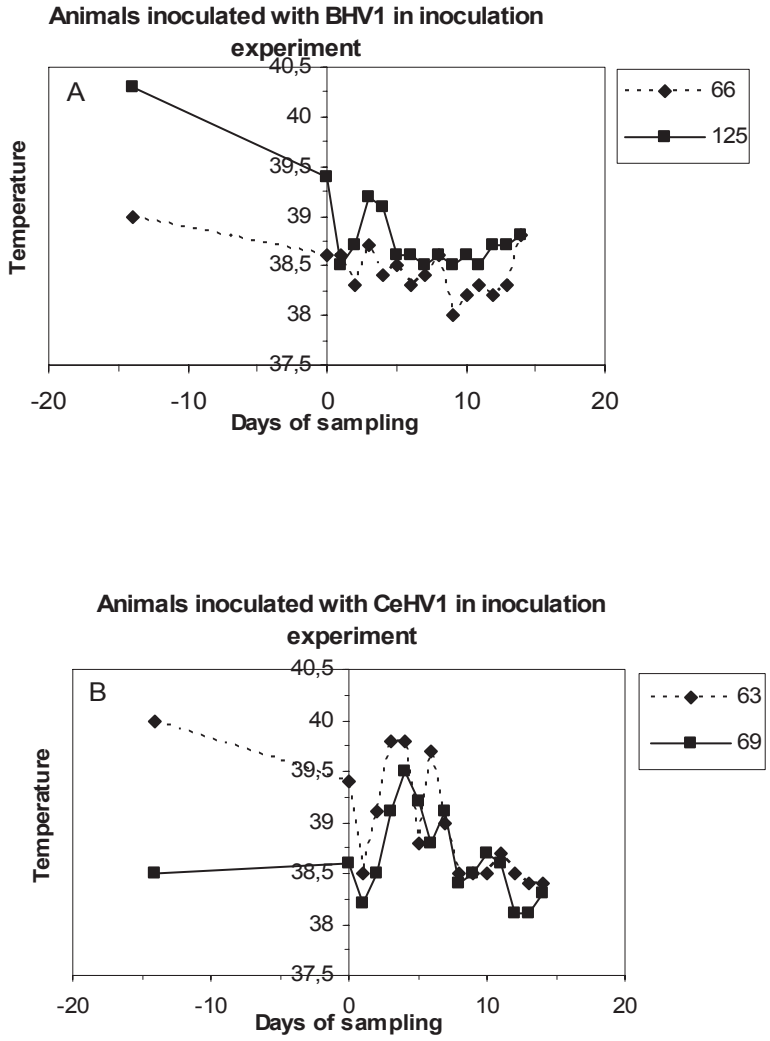

Fig.1. Rectal temperatures recorded in two animals inoculated with BHV1 (A) and in two animals inoculated with CeHV1 (B).

One of the two animals inoculated with CeHV1 showed restless behaviour before D_0 of the experiment, which had probably to do with the acclimatisation of the animal to the housing circumstances. Both animals showed lively behaviour after D_0 of the experiment. At D_7-D_9 and D_{12} after the inoculation with CeHV1 both animals showed some nasal discharge for several days, just after detecting the highest amount of virus. Both animals showed a rise in temperature at the time of virus excretion (Fig. 1B). Thereafter, the temperature dropped to their average value of about 39.0°C and 38.6°C, respectively.

Transmission experiments

During the acclimatisation period, the ten animals of the first transmission experiment showed lively behaviour and the ten animals of the second transmission experiment showed sometimes a bit restless behaviour and did not always eat their daily amount of food. After the acclimatisation period, all animals showed lively behaviour. Some nasal discharge was observed in one animal of the second transmission experiment at four adjacent days before inoculation and in one contact animal of each experiment for one (D_{16}) and two (D_6 and D_8) days, respectively. This was probably not related to an infection with BHV1 because all animals were tested negative in the BHV1 gB ELISA before the start of the experiment. Moreover, no virus was detected in the nasal swabs of the contact animals and none of the contact animals became seropositive to BHV1. The temperature of all animals was elevated at D_0, probably because it was the first time that rectal temperatures were taken. For one inoculated animal of each experiment, the temperature remained relatively high (both mean of 39°C) during the whole experiment compared to the other inoculated animals of the same experiment. For one animal this could be due to the infection with BHV1. Another explanation could be the excitable nature of the animal.

Virus detection and antibody responses

Inoculation experiment

One animal inoculated with BHV1 excreted virus in the range of $10^{1.3}$ to $10^{3.80}$ $TCID_{50}$ per ml of nasal fluid for five days (D_3-D_7) and the other deer excreted virus with a titre of $10^{1.61}$ $TCID_{50}$ per ml of nasal fluid only at day ten (Table 1). One animal became seropositive to BHV1 two weeks after inoculation and the other animal three weeks after inoculation (Fig. 2).

The two animals inoculated with CeHV1 excreted virus in the range of $10^{1.90}$ to$10^{7.3}$ $TCID_{50}$ per ml of nasal fluid for seven (D_1-D_7) and eight days (D_1-D_8), respectively (Table 1). Both animals became seropositive in the BHV1 gB ELISA after one week (Fig. 2). Obviously, this was due to cross-reactivity of anti-CeHV1 gB antibodies with the BHV1 gB antigen in the test. Both animals inoculated with CeHV1 excreted higher virus titres and showed higher blocking

Table 1: Detected virus titres (^{10}log $TCID_{50}$) per ml nasal fluid from day 0 to day 10 in red deer in the inoculation experiment.

Dpi:	0	1	2	3	4	5	6	7	8	9	10
Nr. Red deer											
66^{BHV1}	-	-	-	-	-	-	-	-	-	-	1.46[a]1/2[b]
125^{BHV1}	-	-	-	2.902/2	3.702/2	1.3[c] 0/2	3.802/2	2.902/2	1.32/2	-	-
63^{CeHV1}	-	4.062/2	5.72/2	5.82/2	5.052/2	5.902/2	4.612/2	2.052/2	-	-	-
69^{CeHV1}	-	3.32/2	5.82/2	6.462/2	3.662/2	7.32/2	2.942/2	1.902/2	2.552/2	-	-

Two animals (66 and 125) were inoculated with BHV1 and two animals (63 and 69) were inoculated with CeHV1. Dpi = days post infection; - = virus isolation negative.

[a] The samples that were positive in the virus isolation were titrated once

[b] The first number is the number of times the virus isolation was positive and the second number is the number of times the sample was tested

[c] The sample was negative in the virus isolation but positive in the virus titration

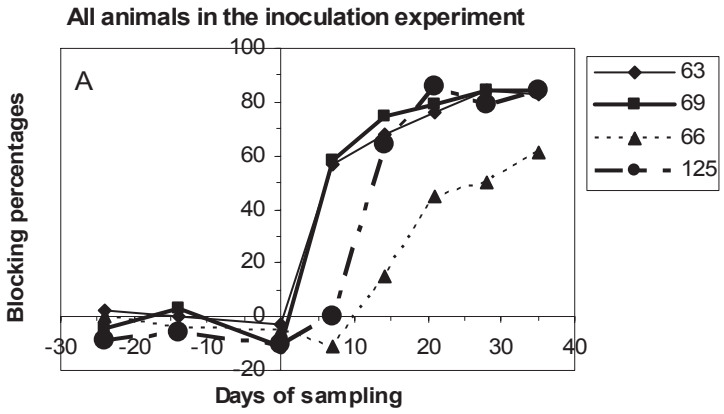

Fig. 2. Blocking percentages measured by the BHV1 gB ELISA of the sera of four animals in the inoculation experiment. Two animals (66 and 125) were inoculated with BHV1 and two animals (63 and 69) were inoculated with CeHV1.

percentages than the two animals inoculated with BHV1.

The results of our inoculation experiment showed that both animals inoculated with BHV1 became infected (excreted virus and seroconverted) and that the BHV1 gB blocking ELISA could be used to detect red deer antibodies against BHV1. Based on this information, it was decided to perform two five-to-five transmission experiments to investigate to what extent BHV1 could be transmitted among red deer.

Transmission experiments

Table 2 shows the detected BHV1 virus titres per ml nasal fluid of the inoculated animals from days zero to five and the presence or absence of antibodies to BHV1. From six out of ten inoculated animals, virus was detected in the nasal secretions in the range of $10^{1.3}$ to $10^{2.94}$ $TCID_{50}$ per ml nasal fluid. Virus was not isolated from any contact animal.

Figure 3 shows the blocking percentages in the gB ELISA for the sera of all animals collected over a six-week period. In each transmission experiment, only one inoculated animal became seropositive to BHV1 after two and one week, respectively (Fig. 3A,B). However, an increase in the blocking percentage for the sera of three other inoculated animals of experiment one to 16%, 31% and 35%, respectively, was found after two weeks and also an increase in the blocking percentage for the sera of four inoculated animals of experiment two to 36%, 14%, 28% and 8%, respectively, was found after two weeks. As all the blocking percentages stayed below 50%, these animals were not considered seropositive.

Table 2: Detected BHV1 virus titres (^{10}log $TCID_{50}$) per ml nasal fluid from days 0 to 5 and the presence or absence of antibodies against BHV1 by the BHV1 gB ELISA in the inoculated deer in the two transmission experiments.

Experiment	Nr. red deer	Virus detection for Dpi 0-5						Antibody responses
		0	1	2	3	4	5	
	118	-	-0/3[a]	-	-0/3	-	-0/3	-
	129	-	-0/3	-	-0/3	-	-0/3	-
I	131	-	**1.48**[b]5/5	-0/3	-0/3	-	-	-
	136	-	**1.3**[b]3/7	**1.3**[b]2/7	-0/3	-0/3	-	+
	140	-	**2.94**[c]5/5	**1.3**[b]2/7	-0/3	-0/3	-	-
	67	-	**1.5**[c]1/2	**1.88**[c]2/2	**1.73**[c]2/2	-0/3	-0/3	+
	76	-	-0/3	**1.3**[c]1/3	-0/3	**2.90**[c]2/2	-0/3	-
II	113	-	-0/3	-	-0/3	-	-0/3	-
	126	-	-0/3	-	-0/3	-	-0/3	-
	138	-	-0/3	-0/3	**2.39**[b]2/2	**2.66**[c]2/2	-0/3	-

In none of the nasal swabs taken from the 10 contact deer BHV1 could be detected and none of the 10 contact deer was considered seropositive to BHV1. Dpi = days post infection; *Virus detection:*

- virus isolation-negative; *Antibody responses*: + detection of antibodies against BHV1 gB;

- no detection of antibodies against BHV1 gB.

[a] The first number is the number of times the virus isolation was positive and the second number is the number of times the sample was tested

[b] The sample was titrated twice, the mean virus titre was taken

[c] The sample was titrated once.

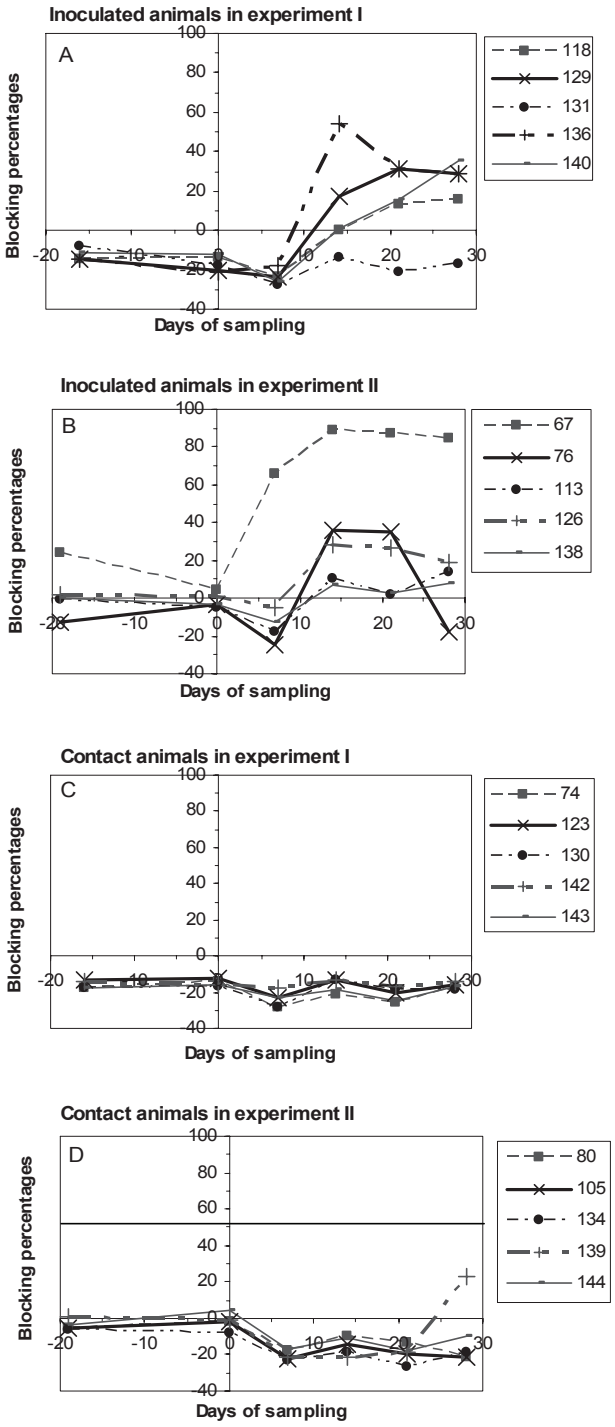

Fig.3. Blocking percentages measured by the BHV1 gB ELISA of the sera of the inoculated animals (A) and the contact animals (C) in the first transmission experiment and of the inoculated animals (B) and the contact animals (D) in the second transmission experiment.

One inoculated animal of the first experiment that excreted virus at day one, did not respond at all in the gB ELISA. The blocking percentages for the sera of the contact animals of the first experiment stayed all below zero (Fig. 3C), and thus, the animals were considered seronegative. In the second experiment, an increase in the blocking percentage for the serum of one contact animal to 23% was found in week four, but this animal was also not considered seropositive (Fig. 3D).

Estimation of the reproduction ratio (*R*) and of the mean infectious period

In each transmission experiment, $S_0 = 7$, $I_0 = 3$ and $C_0 = 0$ at the start of the experiment ($t = 0$) and $S_{28} = 7$, $I_{28} = 0$ and $C_{28} = 3$ at the end of the experiment ($t = 28$ days). The maximum likelihood estimation for R was estimated zero, using the combined likelihood. As the estimated value zero is an extreme value for all possible values of R, it is customary to look only at the one-sided test and the one-sided confidence interval. A one-sided statistical test was performed for which the H_0-hypothesis of $R \geq 1$ was rejected ($P = 0.04$). The one-sided 95% confidence interval for R was calculated, which does not include the value 1 [0.0 – 0.94]. The mean infectious period was estimated at 2 [1.49 – 2.51] days. The arithmetic mean was estimated from the number of days virus was detected in all the infected animals of the two transmission experiments.

Discussion and conclusions

In our inoculation experiment, the two red deer inoculated with BHV1 strain Lam excreted virus for five days (D_3-D_7) and for one day (D_{10}) and seroconverted after two and three weeks, respectively. The two red deer inoculated with CeHV1 excreted virus for seven (D_1-D_7) and eight days (D_1-D_8), respectively, and both deer became seropositive after one week. The animals inoculated with their own herpesvirus (CeHV1) were probably more infectious (higher amounts and more days of virus excretion) than the animals inoculated with BHV1. This was expected based on the experiment of Reid et al. (1986), who also found low BHV1 titers in red deer after inoculation.

When comparing the BHV1 excretion during the inoculation experiment with the BHV1 excretion during the transmission experiments, it was observed that more BHV1 was excreted during the inoculation experiment than during the transmission experiments. Although, the difference is not big, it illustrates that each experiment is subject to influences caused by differences in susceptibility and infectiousness due to natural variation in the course of infection, the age of the animal or the sensitivity to stress.

The maximum likelihood estimation for *R* was estimated zero and the one-sided 95% confidence interval was [0.0 – 0.94]. Below we show the impact on the estimation of *R* for different numbers of infected animals at the start and at the end of each experiment. Three of the inoculated animals that did not excrete virus, did show an increase in the blocking percentage (percentage < 50%) after two weeks, at the same time as the inoculated animals that were considered seropositive (percentage ≥ 50%). The threshold value for the blocking percentage was set at 50% to avoid, for all kinds of technical reasons, that animals not infected with BHV1 were considered seropositive. For example, a blocking percentage of 24% was found for the serum of animal 67 (See Fig. 3B) at D_{-19}, which could not be explained by an infection with BHV1. If however, our interpretation of the test results was too strict and if we would consider those animals that did not excrete virus but did show an increase in the BHV1 gB blocking ELISA (animals 118 and 129 of experiment one and animal 126 of experiment two) also as infected animals, then we would have had nine animals infected by inoculation instead of six. In that case the one-sided 95% confidence interval for *R* would be [0.0 – 0.72]. This resulted thus in an increase of the power of our experiments.

Also one of the contact animals of the second transmission experiment showed an increase of 23% in the blocking percentage at week four and was not considered seropositive for the same reasons. If we also would have considered this contact animal as an infected animal, the reproduction ratio would be estimated at 0.2 and the two-sided 95% confidence interval included the value 1 [0.1 – 1.3]. In that case, the H_0-hypothesis that *R* was above one would not be rejected ($P = 0.07$).

Some remarks have to be made about the estimation of *R*. Notice that if the animals were very stressed during the transmission experiment, stress could have suppressed the immune system of the animals. This suppression of the immune system would then allow higher excretion titers, which would have enhanced the transmission and hence increase the *R*. It would implicate that the *R* we estimated is actually too high. In that case, the experiment would be a 'worse case scenario' and the real *R* should be lower.

It is known for herpesviruses that reactivation and re-excretion can lead to more transmission from an infected animal than only the transmission immediately following the primary infection. Reactivation was not taken into account in this study and therefore our estimation of *R* could have been underestimated. Our estimate for *R* after the primary infection (R_1) is already very low and as a consequence, the estimate for the overall R_0 (R_1 x (1 + average number of reactivation events in a host lifetime) will also be very low. R_0 may exceed one depending on the average number of reactivation events per host lifetime, which is unknown for BHV1 in red deer. If, for example, we take the maximum estimate for R_1 of BHV1 in red deer and an estimate for the reactivation rate of 0.026 per year of BHV1 in cattle (Koeijer et al., 2003)

and a mean host lifetime of, for example, ten years then the R_0 would be 1.2. This would thus be a 'worse case scenario'. For more calculations regarding the effects of reactivation on the population dynamics of herpesviruses see de Koeijer (2003) and Mollema et al. (2005).
In the transmission experiments presented here no BHV1 transmission among red deer was observed. The one-sided 95% confidence interval for R [0.0 – 0.94] shows that limited transmission may occur among red deer. Based on these results, we would expect only minor outbreaks of BHV1 to occur in red deer populations. It can be concluded that most likely red deer will be a satellite group for BHV1. Consequently, it is not necessary for the eradication of BHV1 in cattle to eradicate BHV1 in red deer populations as well.

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References

Anderson, R.M., 1982. Directly transmitted viral and bacterial infections of man. In: Anderson, R.M. (Ed.), Population dynamics of infectious diseases: theory and applications, Chapman and Hall, London, pp. 36.

Bailey, N.T.J. 1975. The mathematical theory of infectious diseases and its applications, second edition, Griffin, London.

Bosch, J.C. 1997. Bovine herpesvirus 1 marker vaccines: tools for eradication? Ph.D. Thesis. University of Utrecht, the Netherlands, pp. 122.

Diekmann, O., Heesterbeek, J.A.P., Metz, J.A.J., 1990. On the definition and computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J. Math. Biol. 28, 365-382.

Dietz, K. 1982. Overall population patterns in the transmission cycle of infectious disease agents. In: Anderson, R.M., May, R.M. (Eds.), Population biology of infectious diseases, Springer-Verlag, Berlin, 87-102.

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpesviruses, Part I, Bovine herpesvirus 1. Vet. Bull. 4**7**, 317-343.

Hage, J.J. 1997. In: Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, the Netherlands, pp. 212.

Jones, C., 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. Clin. Microbiol. Rev. 16, 79-95.

De Jong, M.C.M., Kimman, T.G. 1994. Experimental quantification of vaccine-induced reduction in virus transmission. Vaccine 12, 761-766.

Kaashoek, M.J., Straver, P.H., Van Rooij, E.M.A., Quak, J., van Oirschot, J.T., 1996. Virulence, immunogenicity and reactivation of seven bovine herpesvirus 1.1 strains: clinical and virological aspects. Vet. Rec. 139, 416-421.

De Koeijer, A.A. 2003. Infectious diseases among animals: combining models and data. Ph.D. Thesis. University of Utrecht, the Netherlands, pp. 152.

De Koeijer, A., Nodelijk, G., Mars, M.H., Mourits, M. 2003. Estimation of the reactivation rate of bovine herpesvirus 1 from field data. Report by the Animal Sciences Group, Lelystad, the Netherlands, 03/0026609, 20 p. [in Dutch].

Kramps, J.A., Magdalena, J., Quak, J., Weerdmeester, K., Kaashoek, M.J., Maris-Veldhuis, M.A., Rijsewijk, F.A.M., Keil, G., van Oirschot, J.T., 1994. A simple, specific, and highly sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus 1. J. Clin. Microbiol. 32, 2175-2181.

Kroese, A.H., De Jong, M.C.M., 2001. Design and analysis of transmission experiments. In: Menzies, F.D., Reid, S.W.J. (Eds.), Proceedings of a meeting of the society for veterinary epidemiology and preventive medicine, Noordwijkerhout, the Netherlands, pp. xxi-xxxvii.

Lyaku, J.R.S., Nettleton, P.F., Marsden, H., 1992. A comparison of serological relationships among five ruminant alphaherpesviruses by ELISA. Arch. Virol. 124, 333-341.

Mars, M.H. 2000. In: Vaccinology and epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, the Netherlands, pp. 145.

Mollema, L., de Jong, M.C.M., van Boven, M. 2005. Prolonged persistence of bovine herpesvirus in small cattle herds: a model-based analysis. Epidemiol. Infect. 133, 137-148.

Nixon, P., Edwards, S., White, H., 1988. Serological comparisons of antigenically related herpesviruses in cattle, red deer and goats. Vet. Res. Commun. 12, 355-362.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27, 493-497.

Reid, H.W., Nettleton, P.F., Pow, I., Sinclair, J.A., 1986. Experimental infection of red deer (Cervus elaphus) and cattle with a herpesvirus isolated from red deer. Vet. Rec. 118, 156- 158.

Ros, C., Belák, S., 1999. Studies of genetic relationships between bovine, caprine, corvine, and rangiferine alphaherpesviruses and improved molecular methods for virus detection and identification. J. Clin. Microbiol. 37, 1247-1253.

Thomas, J.C., Tucker, M.J., 1996. The development and use of the concept of a sexually transmitted disease core. J. Infect. Dis. 174 (Suppl. 2), 134-143.

Velthuis, A.G.J. 2002. Quantification of *Actinobacillus pleuropneumoniae* transmission. Ph.D. Thesis. Wageningen University, the Netherlands, pp. 166.


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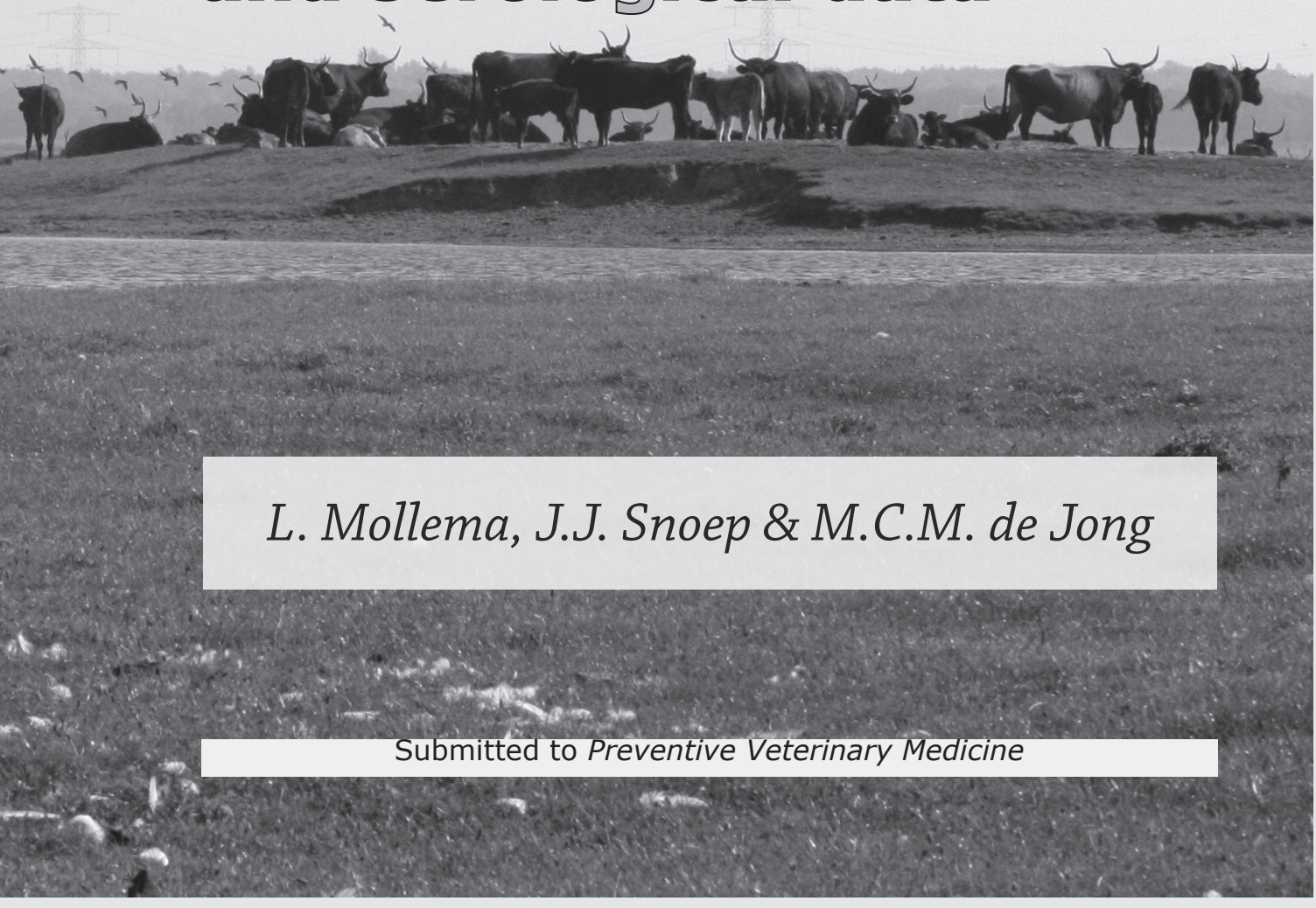
Chapter 5

Dynamics and control of bovine herpesvirus 1 in three feral Heck cattle populations: simulations and serological data



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Summary

Heck cattle are large herbivores and large herbivores are considered to be an important tool for nature conservation and nature development. However, cattle farmers may consider large herbivores in nature reserves to be a risk for the health of their cattle. For example because large herbivores may transmit certain infectious pathogens to their domestic cattle. In this respect, bovine herpesvirus 1 (BHV1) is the most prominent acute problem as cattle farmers want to eradicate BHV1 in their domestic cattle and do not want their domestic cattle to become infected again by BHV1-infected large herbivores.

The dynamics of bovine herpesvirus type 1 (BHV1) in three Heck cattle populations that live in three Dutch nature reserves: 'The Oostvaardersplassen' (OVP), 'Slikken van Flakkee' (SFL) and 'Hellegatsplaten' (HPL) were studied. In all three nature reserves serological data for BHV1 had been gathered. In addition, the data contained also information on the vaccination of part of the Heck cattle populations in SFL (from 1998) and in HPL (from 2000). In order to interpret these serological data a mathematical model was used. It was studied whether in the foreseeable future BHV1 will persist in these Heck cattle populations and whether vaccination of part of the populations will make a difference for this persistence.

The average seroprevalence as observed for BHV1 in respectively OVP, SFL and HPL was 89%, 59% and 49%. The seroprevalence stayed relatively high in OVP whereas it decreased in the two other populations due to vaccination. The mean number of BHV1 outbreaks per year in OVP, using a mathematical model, was estimated at 2.7 (S.E. 0.12), which was about four times higher than the mean number of outbreaks estimated for the two other populations *without* vaccination and fourteen times higher than estimated for the two other populations *with* vaccination. In the model, vaccination also more than halved the average size of the outbreaks. Despite that not all animals were vaccinated, BHV1 was calculated to become extinct in three out of twenty simulations within 15 years. Note that major outbreaks still could take place in the partly vaccinated Heck cattle populations, on average once per 21.4 years. If a major outbreak of BHV1 occurs in those populations then the time to extinction of BHV1 takes a long time, probably longer than desirable for eradication purposes.

Introduction

Heck cattle (Van Vuure, 2005) are a crossbred from various cattle breeds resembling the extinct aurochs *Bos primigenius*. These large herbivores are considered to be an important tool for nature conservation and nature development. In The Netherlands the number of hectares of nature areas that is grazed by large herbivores has increased from 10,000 in 1970 to more than 65,000 in 2002 (Environmental data compendium). Grazing is reintroduced as a natural process in nature reserves where the large herbivores have been present in the past.

Cattle farmers may consider large herbivores in nature reserves to be a risk for the health of their cattle. For example because large herbivores may transmit certain infectious pathogens to their domestic cattle. In this respect, bovine herpesvirus 1 (BHV1) is the most prominent acute problem. A compulsory eradication programme for BHV1 in domestic cattle populations in The Netherlands was started in May 1998. Although this programme is suspended since the end of February 1999, eradication of BHV1 in the domestic cattle populations still takes place on a voluntary basis (Dutch Animal Health Service; Vonk Noordegraaf, 2002). The eradication programme for BHV1 is based on half-yearly vaccination with a marker vaccine of all cattle older than three months. A cattle herd could obtain the BHV1-free status if individual blood tests showed that the herd was free of BHV1, or after removal of the last seropositive cattle. The free status of the certified BHV1-free herds was monitored by monthly bulk-milk tests on dairy herds and half-yearly serological sampling on non-dairy herds (Vonk Noordegraaf, 2002). Farmers thus try to become certified for BHV1-free cattle and do not want their domestic cattle to become infected again by BHV1-infected feral cattle.

BHV1 is an alphaherpesvirus that infects cattle causing infectious bovine rhinotracheitis and genital infections (Gibbs and Rweyemamu, 1977). Once individuals are infected with a herpesvirus they remain carriers of the virus for life (Gibbs and Rweyemamu, 1977; Ackermann et al., 1982) and, under certain stress conditions the virus can reactivate and the carrier hosts become infectious again (Ackermann et al., 1982; Sheffy and Rodman, 1973; Dennett et al., 1976; Hage, 1997; reviewed by Jones, 2003). Infectious carrier hosts may then establish primary infections in susceptible animals (Hage, 1997). If not all carrier hosts have died or have been taken out of that population before the virus is transmitted, BHV1 may persist in the population.

Heck cattle have been introduced into Dutch nature reserves from 1983 onwards (Vulink, 2001). There are three Heck populations living in three Dutch nature reserves. One large population of about 600 animals lives in the nature reserve 'The Oostvaardersplassen' (OVP) and two smaller populations of about 100 animals live in the nature reserves 'Slikken van

Flakkee' (SFL) and 'Hellegatsplaten' (HPL) (Cornelissen and Vulink, 1995; Nieuwdorp, 2000; Van Tienhoven, 2000; Vulink, 2001). Monitoring of the health status of Heck cattle with regard to certain specific diseases takes place each year (Hessels, 1997; Dutch Animal Health Service). This is necessary in order to guard the health of the Heck cattle but also to prevent risks of possible disease transmission to domestic cattle populations. One of the infectious agents that are monitored for, is BHV1.

Until 1995 parts of the area of nature reserves were also used for summer grazing by domestic cattle (Hessels, 1997; Vulink, 2001) and direct contact could have taken place between domestic and feral cattle populations. Nowadays no direct contact can take place between both cattle populations. Probably the only possible transmission route for BHV1 between domestic and feral cattle populations would be via distance related transmission.

Because little is known about BHV1 in feral cattle populations, the dynamics of BHV1 in the three feral Heck cattle populations that were already mentioned above, were studied. Data on the transmission of an infection within wildlife populations are scarce, therefore the scarce serological data on BHV1 in the Heck cattle populations were combined with model simulations of the dynamics of a BHV1 infection. The simulations were used to give insight in how often outbreaks of BHV1 do occur in the Heck cattle population, the mean size of an outbreak, the mean age at infection and the time to extinction. As the available dataset includes data on vaccination of part of the feral cattle population in the nature reserves SFL and HPL, the effects of vaccination on the dynamics of BHV1 in these populations could be estimated.

Material and Methods

Monitoring

Monitoring of the health status of Heck cattle with regard to certain specific diseases takes place each year (Hessels, 1997; Dutch Animal Health Service). The monitoring for the presence of infectious pathogens in the Heck cattle population in OVP consists of i) gathering of ten fresh fecal samples twice per year, which were tested for the presence of coccidiosis and eggs of worms per gram faeces; ii) post-mortem examination of animals by the Dutch Animal Health Service; and iii) testing of sera for the presence of antibodies against brucellosis, leptospirosis, infectious bovine rhinotracheitis, bovine leukosis, paratuberculosis, liver fluke, bovine virus diarrhoea and salmonellosis (i.e. in the case that carcasses were fresh and some blood could be taken) (Hessels, 1997). Each year about twenty animals were offered to the Dutch Animal Health Service for monitoring the health status of the Heck cattle in OVP. If in that year not enough animals died then animals with a low body index score or older (>3

years) female animals were shot to have enough animals for the monitoring at the end of the year (Dutch Animal Health Service). The Heck cattle in the other two nature reserves SFL and HPL were tested once per year for the presence of antibodies against bovine leukosis, leptospirosis, paratuberculosis, infectious bovine rhinotracheitis and brucellosis. Only the animals that could be caught were tested. The animals in SFL and HPL were also vaccinated, which occurred at the same time a blood sample of the animal was taken.

Serological data

Serological data of BHV1 were gathered in three different Heck cattle populations living in three nature reserves in The Netherlands namely, OVP, SFL and HPL. For the interpretation of the serological data we briefly describe below the three different natures reserves with their Heck cattle populations and the data that were gathered.

'The Oostvaardersplassen' (OVP)

The 5600 hectares nature reserve is an eutrophic wetland in the central part of The Netherlands and is an enclosed area of which 2000 hectares is grazed by large herbivores. In OVP there is a year-round grazing regime of Heck cattle and Konik Horses - a primitive breed of horse, originating from Poland - since 1984. In 1992 red deer (*Cervus elaphus*) were introduced (Vulink, 2001). The Heck cattle population consisted of a population of about 320 animals (in 2002) in the eastern part of the nature reserve and a population of about 280 animals (in 2002) in the western part of the nature reserve.

Limited serological sample data from the whole Heck cattle population were present from 1997 till 2003. In total 132 non-random blood samples were taken from dead animals. The number of blood samples per year ranged from 14 to 28. The age of the dead animals could not be estimated in all cases. Therefore the total number of blood samples could vary from the number of blood samples when adding up the blood samples per age-category. Of animals between 0-1 year old (also including 1 year) in total 20 blood samples were taken, 25 blood samples were taken of animals between 1-2 years old (also including 2 years) and 81 blood samples were taken of animals older than 2 years. When the animals arrived at the Dutch Animal Health Service, blood was taken from the heart. No vaccination against BHV1 took place in this Heck cattle population. The test that was used to detect antibodies against BHV1 for screening was the BHV1 gB Blocking ELISA (Kramps et al., 1994) and for confirmation a Danish test system - consisting of a blocking ELISA and an indirect ELISA - was used (de Wit, 1998).

'Slikken van Flakkee' (SFL)

The nature reserve SFL is an enclosed area of about 730 hectares large and is situated in the Southwest of The Netherlands below the peninsula 'Goeree-Overflakkee'. SFL became permanently drained after the closure of the lake called the 'Grevelingen' in 1971. In SFL lives a Heck cattle population of approximately 100 animals. There is a year-round grazing regime of Heck cattle and Fjord horses since 1983 (Van den Tempel, 1987; Cornelissen and Vulink, 1995).

Part of the Heck cattle population was vaccinated once a year against BHV1 during the winter period, starting in 1998. The newborn calves, which were born after vaccination had taken place, were attempted to vaccinate the following winter period. It could also occur that some animals could not be caught for the yearly vaccination campaign. These animals were then also attempted to vaccinate the following winter period. The fraction of the Heck cattle population that was vaccinated probably lies in between 70% and 80%. The vaccine used was Bayovac IBR marker vivum: an attenuated live gE negative vaccine (Mars, 2000). The BHV1 gE Blocking ELISA (Kaashoek et al., 1994; Van Oirschot et al., 1997) was used to detect antibodies against BHV1 and to distinguish between animals infected with the wild BHV1 type or with the BHV1 vaccine. Besides vaccination, also BHV1-seropositive cattle were removed from the population.

Serological sample data were present from 1997 and from 2000 till 2004. In total 443 blood samples were taken. The number of blood samples per year ranged from 15 to 128. Of animals between 0-1 years old in total 41 blood samples were taken, 75 blood samples were taken of animals between 1-2 years old and 310 blood samples were taken of animals older than 2 years.

'Hellegatsplaten' (HPL)

The nature reserve HPL is an area containing patches of land and water. The area is about 322 hectares large. The reserve is an enclosed area and is situated on the peninsula 'Goeree-Overflakkee' in the Southwest of The Netherlands. Two fresh water lakes, the land of 'Goerree-Overflakkee' and a dam bound the nature reserve. In the past the area was exposed to large differences between low and high tide. Since the construction and closure of the two dams in 1987, the area has changed a lot. In HPL lives also a Heck cattle population of approximately 100 animals. In this nature reserve there is a year-round grazing regime of Heck cattle and Fjord horses since 1993 (Van Tienhoven, 2000).

Probably the same fraction of the Heck cattle population as in the case of SFL was vaccinated once per year, which started in 2000. Also the same vaccine and the same diagnostic test

were used as in the case of SFL. Also in HPL BHV1-seropositive animals were removed from the population.

Serological sample data of the Heck cattle population were present from 1993 and from 1998 till 2004. In total 551 blood samples were taken. The number of blood samples per year ranged from 25 to 132. Of animals between 0-1 years old in total 59 blood samples were taken, 90 blood samples were taken of animals between 1-2 years old and 320 blood samples were taken of animals older than 2 years.

Simulation model

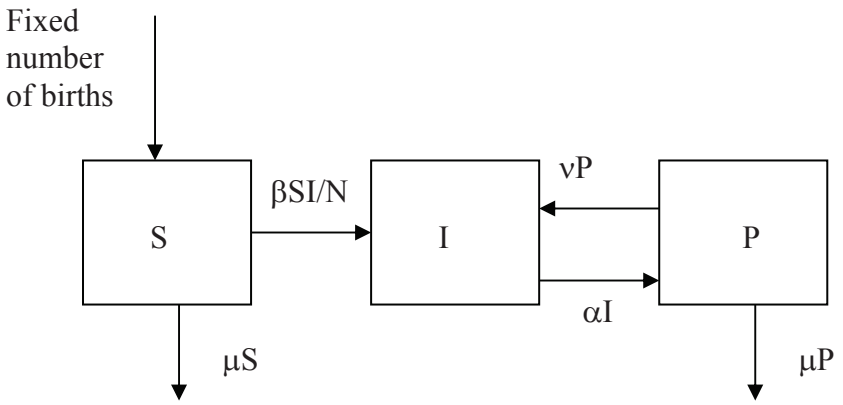
We used a modification of the simulation model by Mollema et al. (2005), programmed in Borland Delphi 2005, to estimate the mean age at infection, the mean number of outbreaks per year, the mean size of an outbreak and the mean time between two outbreaks. Figure 1 shows a flow diagram of the model. A full description of the model is given in Mollema et al. (2005). The modifications of this model were mentioned below.

It was assumed that each year the same number of animals was born and the timing corresponded to the birth peak observed for the Heck cattle population in the nature reserves. Each year most Heck cattle were born between February and May (Platteeuw et al., 2000). The host lifespan was an exponentially distributed host lifespan with a fixed maximum age (i.e. a truncated exponentially distributed host lifespan). For computational efficiency we cut off the maximum age of an animal at 18 years. The effect of this was that the time to extinction was somewhat lower than in the case of an exponentially distributed host lifespan and somewhat higher than in the case of a fixed host lifespan (see Mollema et al., 2005).

The overall reproduction ratio (R_0) is the average number of newly infected individuals caused by one typical infected individual during its lifetime in a fully susceptible population. R_0 for BHV1 is equal to the reproduction ratio of a single outbreak (R_1) plus the expected number of times reactivation events take place per host lifetime (ν/μ) times the reproduction ratio of a single outbreak ($R_0 = \left(1 + \frac{\nu}{\mu}\right) R_1$).

The reproduction ratio of a single outbreak for BHV1 in a vaccinated population was denoted by R_v. The reproduction ratio of a single outbreak (R_1) in a partly vaccinated population was $fR_1 + (1\text{-}f)R_v$, assuming separable mixing (see also Diekmann and Heesterbeek, 2000), where f is the fraction of non-vaccinated animals in the population. From experimental studies it was known that the marker vaccine that is used to eradicate BHV1 in the Heck cattle populations in SFL and HPL reduced the primary spread of BHV1 but did not effect the amount of virus

spread after reactivation (Bosch, 1997; Mars, 2000). Further it was not shown from literature that vaccination also reduced the reactivation rate (Mars, 2000). In the model it was therefore assumed that the vaccine only reduced the reproduction ratio by reducing the infectivity of the infectious animals. The simulations were repeated ten times for each parameter combination. Two sets of parameter values were used, namely one set of parameter values based on the situation in OVP and one set of parameter values based on the situations in SFL and in HPL, which were comparable. The simulations were stopped after t = 15 years or when no carrier individuals were left in the population. For SFL and HPL *with* vaccination another ten simulations were done. These ten simulations were used for studying the effect of vaccination on the mean age at infection. The time-step in the model was chosen such that the probability at two events occurring at the same time was always smaller than 0.01.



S = number of susceptible individuals; I = number of infected and infectious individuals; P = number of carrier individuals; α = recovery rate (time^{-1}); β = transmission rate constant; μ = mortality rate (time^{-1}); ν = reactivation rate (time^{-1})

Fig.1. A flow diagram of the epidemiological *SIP* model as was also used in the study of Mollema et al. (2005). A full description of the model is given in Mollema et al. (2005).

Parameter values

Parameter values were derived from data of both feral and domestic cattle populations.
In the case of OVP we started with 320 animals (eastern subpopulation) of which was assumed that 220 animals were carriers for BHV1 and 100 animals were susceptible (quasi-stationary state for $R_1 = 3.2$). Each year about 60 newly susceptible animals were born (age = 0) in the population. The counts for the number of animals in the eastern subpopulation were done during winter-spring in 2002. The age of all carrier animals and of 40 (100-60) susceptible animals was chosen at random. The mortality rate was set at 0.17 $year^{-1}$, which resulted each year in about a same number of dead animals as newborn animals. The maximum age of an animal was set at 18 years as was explained earlier.
Because there were no data available on the dynamics of BHV1 within feral cattle populations, data from field studies were taken, describing the dynamics of BHV1 within domestic dairy cattle herds in The Netherlands (Bosch, 1997). The reproduction ratio (R_1) was estimated at 3.2 (Bosch, 1997). The reactivation rate was calculated from data of field studies done by Bosch (1997) and was estimated at 0.026 (95% CI: 0.012-0.047) $year^{-1}$ (De Koeijer et al., 2003).
SFL and HPL were managed in such a way that there were about 100 animals of which about 30 animals were bulls and about 70 animals were cows. We started with 100 animals of which it was assumed that 69 animals were carriers for BHV1 and 31 animals were susceptible (quasi-stationary state for $R_1 = 3.2$). Twenty-three out of those 31 susceptible animals were newborn animals (age = 0). The age of all carrier animals and of 8 (31-23) susceptible animals was chosen at random. Every year the animals that were present at the moment vaccination took place were vaccinated. Vaccination took place at the beginning of each year, starting at $t = 0$. Culling of seropositive animals was not taking into account in the model. The mortality rate was set at 0.21 $year^{-1}$ and the maximum age of an animal at 18 years. The reproduction ratio (R_1) of the non-vaccinated animals and the reactivation rate had the same values as for OVP. The reproduction ratio (R_1) for BHV1 in the vaccinated animals was estimated at 1.2 (Mars, 2000) from data from field studies describing the dynamics of BHV1 within vaccinated domestic dairy cattle herds in The Netherlands.

Mean age at infection

The mean age at infection was estimated from simulation data for OVP and for SFL and HPL *without* vaccination. The mean age at infection could not be estimated for SFL and HPL *with* vaccination due to that there was no quasi-stationary state yet. However, the effect of yearly vaccination of part of the Heck cattle population on the age at infection could be shown in Figure 3.

The mean age at infection followed from seroprevalence data in a certain age-class: [0-1⟩, [1-2⟩, …, [17-18⟩. The mean of each age-class was taken for which the seroprevalence was estimated from simulation data. The mean age at infection (*A* or $\frac{1}{\lambda}$) was then calculated from $w(a) = 1 - e^{-\lambda a}$ (Dietz, 1975), where *w*(*a*) is the observed fraction of positive animals at a certain age *a*. For the calculation of the mean age at infection it was assumed that there was a quasi-stationary state and that the death rates were age-independent (i.e. an exponential age distribution). The mean age at infection could also be estimated by:

$A = \frac{L}{R_0 - 1}$ (Dietz, 1975), where *L* is the mean host lifetime (i.e. the life expectancy at birth) and R_0 is the overall reproduction ratio. If the mean age at infection became larger than the mean host lifetime by vaccinating the population, then the reproduction ratio became less than one. If $R_0 < 1$ only minor outbreaks will take place in the population. If $R_0 > 1$ then minor and major outbreaks can take place. The mean host lifetime (*L*) of the Heck cattle population was estimated by $L = \int_0^{18} a\mu e^{-\mu a} da$, where μ is the mortality rate and *a* is the age of the animal. For computational efficiency we cut off the maximum age of an animal at 18 years as was explained earlier.

Results

Serological data

Figure 2 shows the overall seroprevalences and the seroprevalences for three different age-categories (0-1 year; 1-2 years; >2 years) as observed for BHV1 per year for all three nature reserves. The observed seroprevalence for BHV1 in OVP was on average 89% and fluctuated between 63% and 100%. The largest fluctuations in seroprevalence between the years and the lowest average seroprevalence were found in the youngest animals.

In SFL vaccination of part of the Heck cattle population started in 1998. Serological data in the pre-vaccination period were only present from 1997. In that year the seroprevalence was 87%. Note this estimate was based on only 15 blood samples. The average seroprevalence after vaccination was 58%. The seroprevalence for BHV1 in SFL stayed relatively high after vaccination, which was probably due to outbreaks that have occurred (see simulation results below). The average seroprevalence of animals between 0-1 years old was about 29%, of animals between 1-2 years old about 20% and of animals older than 2 years about 72%. In total 198 different animals have been tested of which 154 animals have been tested several times. Seventeen (9%) animals have first been tested negative and thereafter positive, which

could be due to that animals were not fully protected as the animals were only vaccinated once per year or due to logistic and/or administrative mistakes. 13 (7%) animals have first been tested positive and thereafter negative, which could be due to logistic and/or administrative mistakes.

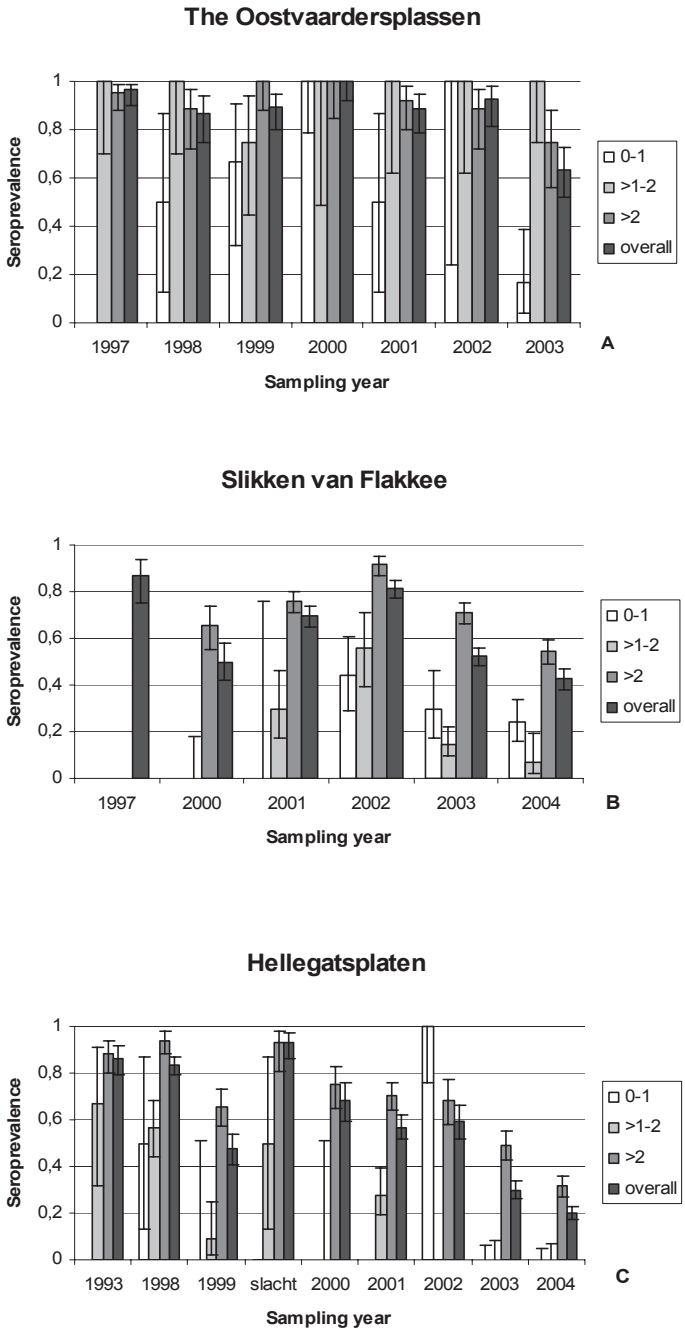


Fig.2. Overall seroprevalences and seroprevalences for three different age-categories (0-1 year; 1-2 years; >2 years) as observed for BHV1 per sampling year for the nature reserves 'The Oostvaardersplassen' (Fig. 2A), 'Slikken van Flakkee' (Fig. 2B) and 'Hellegatsplaten' (Fig. 2C).

Vaccination of part of the Heck cattle population started in 2000 in HPL. Before vaccination the average seroprevalence was 76% and fluctuated between 48% and 93%. After vaccination the average seroprevalence was 37%. A relatively high decrease was shown before vaccination between 1998 and 1999 (Fig. 2C) after which the seroprevalence increased again. The seroprevalence decreased slowly after vaccination had taken place. Note the average seroprevalence after vaccination was lower in HPL as compared to SFL whereas vaccination had started two years later in HPL. This could be for example due to that several outbreaks had taken place in SFL or due to that the seroprevalence was higher in SFL at the start of vaccination. Further note that in 2003 and 2004 all animals aged between 0-1 year old and between 1-2 years old have been tested negative in HPL. The average seroprevalence of animals between 0-1 years was about 2%, of animals between 1-2 years old about 17% and of animals older than 2 years about 62%. In total 216 different animals have been tested of which 151 animals have been tested several times. Eleven (5%) animals have first been tested negative and thereafter positive (of which 9 animals were probably infected because vaccination was not yet applied) and 12 (6%) animals have first been tested positive and thereafter negative due to reasons explained earlier.

Simulation model

In three out of twenty simulations for SFL and HPL *with* vaccination the BHV1 infection chain had stopped before $t = 15$ years due to that no carrier individuals were present anymore in the population. In these partly vaccinated populations, BHV1 had thus already become extinct within 15 years.

Mean age at infection

The mean ages at infection for BHV1 were estimated from simulation data as was explained earlier. The mean age at infection for OVP was 1.7 (S.E. 0.05) years and the mean host lifetime was 4.8 years. For SFL and HPL *without* vaccination the mean age at infection was 2.5 (S.E. 0.27) years and the mean host lifetime 4.2 years.

Figure 3 shows for the simulated data (ten runs) an increase in seroprevalence for an increasing age in the case of OVP (Fig. 3A) and in the case of SFL and HPL *without* (Fig. 3B) and *with* (Fig. 3C) vaccination. The faster increase in seroprevalence for an increasing age for OVP (Fig. 3A) compared to SFL and HPL *without* vaccination (Fig. 3B) could be explained by that on average more outbreaks might take place in OVP due to that more carrier animals were present in OVP as compared to SFL and HPL. Figure 3B then shows that the increase in seroprevalence for an increasing age was faster *without* vaccination than *with* vaccination (Fig. 3C).

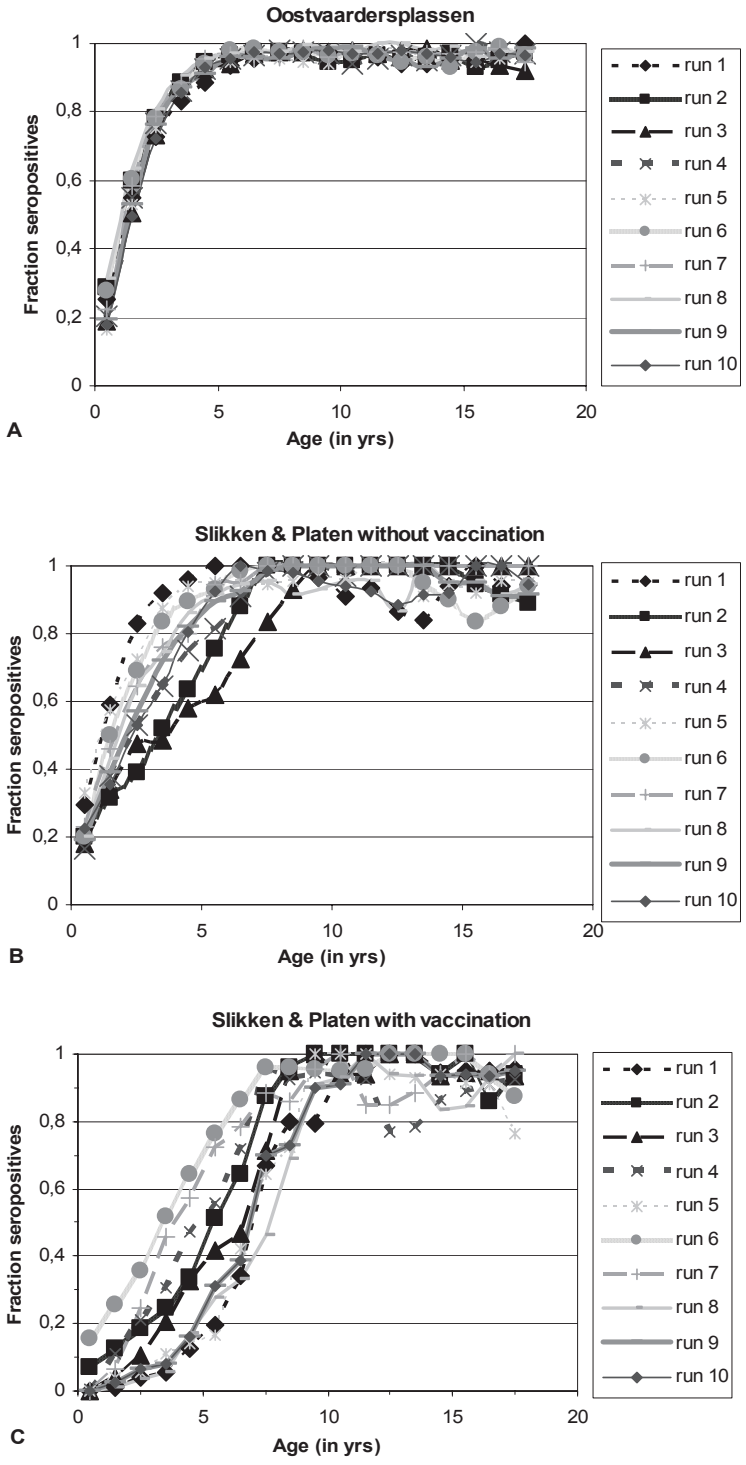


Fig.3. Seroprevalences at a certain age estimated, using a simulation model, for the nature reserves 'The Oostvaardersplassen' (Fig. 3A), 'Slikken van Flakkee and Hellegatsplaten' *without* vaccination (Fig. 3B) and 'Slikken van Flakkee and Hellegatsplaten' *with* vaccination (Fig. 3C). The simulations were repeated ten times (ten runs) for each parameter combination. The simulations were stopped after t = 15 years or when no latently infected individuals were left in the host population.

It took on average more years for a susceptible animal and a vaccinated animal not infected with the wild type of BHV1, to become infected. Also a smaller fraction of susceptible animals and vaccinated animals not infected with the wild type of BHV1, would become infected in each outbreak *with* vaccination than *without* vaccination.
Both the ranges of the seroprevalences at a certain age were wide for SFL and HPL *with* and *without* vaccination, which could be explained by the relatively small population size and the number of simulation runs. Another explanation of the wide range of seroprevalences at a certain age in the case of SFL and HPL *with* vaccination (Fig. 3C) was that the simulation started with hundred individuals in a quasi-stationary state for $R_1 = 3.2$ and due to vaccination the system was changing towards a quasi-stationary state for $R_1 = 1.2$, which would take some time.

Mean number of outbreaks, mean size of an outbreak and mean time between two outbreaks
Table 1 gives the estimates for the mean number of outbreaks per year, mean size of an outbreak and the mean time between two outbreaks in the case of OVP and in the case of SFL and HPL *with* and *without* vaccination. The mean number of outbreaks was estimated at 2.7 (S.E. 0.12) per year in OVP, which was about fourteen and four times higher as compared to SFL and HPL *with* and *without* vaccination, respectively. This could again be explained by that the total probability of reactivation (i.e. introduction of BHV1) was much higher in OVP as compared to SFL and HPL. The reactivation rate is the same in both populations but the number of carrier animals in OVP is much higher than in SFL and HPL. Table 1 further shows that in the model, vaccination more than halved the average size of an outbreak. On average once per 5.4 years an outbreak causing less than 10% newly infectious animals might occur in SFL and HPL *with* vaccination compared to once per 2.7 years in a non-vaccinated population. Once per 21.4 years an outbreak causing between 10% and 50% newly infectious animals might occur in the vaccinated population compared to once per 4.3 years in the non-vaccinated population. On average no outbreak causing more than 50% newly infectious animals might take place in the vaccinated population compared to once per 13.6 years in the non-vaccinated population.
Each year about 2.25 outbreaks causing less than 10% newly infectious animals might occur in OVP. Once per 2.4 years an outbreak causing between 10% and 50% newly infectious animals might occur and on average once per 150 years an outbreak causing more than 50% newly infectious individuals might take place in OVP.

Table 1: Estimates from simulation data for the mean number of outbreaks per year, the mean size of the outbreaks and the mean time between two outbreaks (S.E.) for OVP and SFL and HPL *with* and *without* vaccination

	Oostvaardersplassen	Slikken van Flakkee and Hellegatsplaten	
		Vacc.	*Non-vacc.*
Mean number of outbreaks per year	2.7 (0.16)	0.2 (0.05)	0.7 (0.06)
Mean size of outbreaks	15.8 (1.4) (range: 1-154)	6.8 (1.49) (range: 1-38)	18.4 (2.20) (range: 1-91)
Mean time between two outbreaks (in yrs)	0.4 (0.02)	3.5 (0.91)	1.4 (0.10)

Discussion

The average seroprevalence as observed for BHV1 in OVP was 89%. Only limited serological data was present for estimating the seroprevalence per year and samples were also not taken at random. Note that blood samples of animals in OVP were taken to monitor whether those animals were free of antibodies against the diseases mentioned earlier and thus not to estimate the seroprevalence for BHV1. For the monitoring less samples were needed and samples of older female animals were preferred. Despite the limited data, it could be concluded that BHV1 was present in the Heck cattle population from 1997 till 2003 and that the observed seroprevalence for BHV1 did not show a significant decrease. Other non-vaccinated feral cattle populations in which BHV1 was also found to be present were, European bisons in Poland (8 out of 66, 12%) (Kita and Anusz, 1991), American bisons (29 out of 76, 38%) (Taylor, 1997) and buffalo in Zimbabwe (30%) (Anderson and Rowe, 1998). The seroprevalence of BHV1 in these feral cattle populations was however much lower as compared to the Heck cattle population in OVP. This could be explained by various reasons such as for example differences in: i) population sizes and population densities; ii) susceptibility and infectivity to BHV1 of the animals; iii) subtypes of BHV1 that are circulating; or iv) times since BHV1 was introduced.

The BHV1 seroprevalence in the SFL and HPL was still decreasing. In 2005 the observed BHV1 prevalence was 35% (42 out of 120) in SFL and 11% (14 out of 123) in HPL (Snoep, data from Dutch Animal Health Service) whereas in 2004 it was estimated at 43% and 20%, respectively. In 2006 the observed BHV1 prevalence was decreased again to 8% (7 out of 87) in HPL (Snoep, data from Dutch Animal Health Service). No data was available yet for SFL in 2006. The serological data contained a relatively high percentage of animals that were first

tested negative and thereafter tested positive and also the other way around. Reasons that could explain these findings were already given in the section Results. It should however be mentioned that the test that was used, the BHV1 gE blocking ELISA (Van Oirschot et al., 1997) has a very high specificity (99%) and also a high sensitivity (98%). Therefore the results that animals were first tested positive and thereafter tested negative and also the other way around were not likely to be due to the diagnostic test that was used. The BHV1 gE blocking ELISA was of course developed to test the presence of antibodies against the wild type of BHV1 in domestic cattle that are vaccinated with the live gE-negative vaccine and not for use in feral Heck cattle. But, as Heck cattle are taxonomically very close to domestic cattle, these diagnostic tests would probably work equally well in feral Heck cattle.

The seroprevalence estimated from the model simulations for the Heck cattle population in OVP also stayed relatively high like the observed BHV1 seroprevalence that was estimated from the serological data. Mollema et al. (2005) calculated that in a demographically stable non-vaccinated population of 50 animals the time to extinction was already in the order of million years. It should be mentioned that the reactivation rate and the host lifespan in this study were estimated at 0.029 $year^{-1}$ and 5.9 years respectively, whereas these parameters were estimated at 0.09 $year^{-1}$ and 10 years respectively, in the study of Mollema et al. (2005). Despite these lower estimates for the reactivation rate and the host lifespan, BHV1 will persist in the Heck cattle population in OVP given its total population size of 600 animals.

From the model simulations it was clear that in three out of twenty partly vaccinated populations the virus became already extinct within fifteen years. In the other partly vaccinated populations only a small percentage of carrier animals was left in the population at the end of the simulation. Because culling of seropositive animals was not taking into account in our model, it was expected that BHV1 would be eradicated sooner in SFL and HPL than estimated from our model. It can be concluded that vaccination of part of the Heck cattle population might be an effective tool for BHV1 eradication in relatively small cattle populations such as the Heck cattle populations in SFL and HPL. However, it has to be taken in mind that major outbreaks might still take place if the reproduction ratio for BHV1 in the vaccinated animals is above 1 as was the case in our model.

Given the results above, could BHV1 also be eradicated in the Heck cattle population in OVP using vaccination? This Heck cattle population is much larger compared to the other two Heck cattle populations. Therefore, it would be more difficult to catch and vaccinate enough animals in order to reduce the effective reproduction ratio to a value less than one. An option for eradication of BHV1 in the Heck cattle population in OVP might be by means of mass immunisation with an oral vaccine. However an oral vaccine for BHV1 is not available at this moment and another problem is that feeding of the animals is considered an undesirable

intervention with the natural developments in the nature reserve. Another option might be replacing part or the whole infected population by a new susceptible and vaccinated population but that is of course a very drastic intervention. Therefore, one could start for example with catching a group of animals from the population, vaccinate those animals and release those animals in the population again. Model simulations could give insight in how long it then would take on average for the Heck cattle population to become BHV1-free.

Should eradication of BHV1 in the Heck cattle population take place anyway? For the eradication of BHV1 in domestic cattle, BHV1 should only be eradicated in the Heck cattle population in OVP if transmission of BHV1 occurs from the Heck cattle population to the domestic cattle populations. In this study it was estimated that on average 2.7 BHV1 outbreaks might yearly take place in this population. Concurrent with transmission within the feral Heck cattle populations also other domestic cattle populations are at risk. The probability of BHV1 transmission to a nearby domestic cattle herd within a radius of 1 km of a previously certified BHV1-free domestic cattle herd with a confirmed BHV1 outbreak, was already calculated to be 5% (Holzhauer et al., (not published)). This probability was then extrapolated to a probability of BHV1 transmission to nearby domestic cattle herds from the Heck cattle population by taking into account the mean number of outbreaks per year that might occur in OVP. This probability was estimated at 13%, which meant that if eight herds were situated within a radius of 1 km of OVP that each year on average one domestic cattle herd might become infected. However, only one domestic cattle herd lies within a radius of 1 km of OVP (Griekspoor, pers. commun.). For this one domestic herd the result meant that this herd might become infected with BHV1 on average once every eight years. No data was available of the BHV1 status of this one domestic herd for the past years for comparison. It should be noted that the estimated probability of BHV1 transmission from the Heck cattle population in OVP to nearby domestic cattle herds has to be interpreted carefully as the actual probability will probably be lower than estimated here because:

1. The disease dynamics of BHV1 in Heck cattle have been assumed to be similar as in domestic cattle but this does not need to be the case. Disease caused by BHV1 has never been reported in Heck cattle whereas it is seen in domestic cattle.
2. The average outbreak size has been estimated to be two or three times smaller in the Heck cattle population than in the domestic cattle populations with a confirmed BHV1 outbreak calculated from the study of Holzhauer et al. (not published).

Eradication of BHV1 in the Heck cattle population would therefore probably not be necessary because it seems that BHV1-infected Heck cattle are not a direct threat to the eradication of BHV1 in domestic cattle. However, more research on to what extent circulation of BHV1 in Heck cattle populations possess a risk to domestic cattle farms has to be done.

Conclusion

The average seroprevalence as observed for BHV1 in respectively OVP, SFL and HPL was 89%, 59% and 49%. The seroprevalence stayed relatively high in OVP whereas it decreased in the two other reserves due to vaccination. The mean number of BHV1 outbreaks per year in OVP, using a mathematical model, was estimated at 2.7 (S.E. 0.12), which was about four times higher than the mean number of outbreaks estimated for the two other populations *without* vaccination and fourteen times higher than estimated for the two other populations *with* vaccination. In the model, vaccination also more than halved the average size of the outbreaks. Despite that not all animals were vaccinated, BHV1 was calculated to become extinct in three out of twenty simulations within 15 years. Note that major outbreaks still could take place in the partly vaccinated Heck cattle populations, on average once per 21.4 years. If a major outbreak of BHV1 occurs in those populations then the time to extinction of BHV1 takes a long time, probably longer than desirable for eradication purposes.

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References

Ackermann, M., Peterhans, E., Wyler, R., 1982. DNA of bovine herpesvirus type 1 in the trigeminal ganglia of latently infected calves. Am. J. Vet. Res. 43, 36-40.

Anderson, E.C., Rowe, L.W., 1998. The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, rift valley fever, ephemeral fever and bluetongue and to *Leptospira* sp. in free-ranging wildlife in Zimbabwe. Epidemiol. Infect. 121, 441-449.

Bosch, J.C., 1997. Bovine Herpesvirus 1 marker vaccines: tools for eradication? Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 122.

Cornelissen, P., Vulink, J.Th., 1995. Grazing in young wetlands. Flevobericht 367, pp. 121 [in Dutch].

Dennett, D.P., Barasa, J.O., Johnson, R.H., 1976. Infectious bovine rhinotracheitis virus: studies on venereal carrier status in range cattle. Res. Vet. Sci. 20, 70-83.

Diekmann, O., Heesterbeek, J.A.P., 2000. What is contact? In: S. Levin (ed.), Mathematical epidemiology of infectious diseases. John Wiley & Son, LTD, Chichester, pp. 153-169.

Dietz, K., 1975. Transmission and control of arbovirus diseases. In: Ludwig, D. & Cooke, K.L. (eds.), Epidemiology. SIAM, Philadelphia, pp. 104–106.

Dutch Animal Health Service (Gezondheidsdienst voor Dieren), Deventer, The Netherlands (http://www.gd-dieren.nl).

Environmental data compendium (Milieu- en natuur compendium), Bilthoven, The Netherlands (http://www.mnp.nl).

Gibbs, E.P.J., Rweyemamu, M.M., 1977. Bovine herpesviruses, Part I, Bovine herpesvirus 1. Vet. Bull. 47, 317-343.

Hage, J.J., 1997. Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 212.

Hessels, A., 1997. Protocol to determine, guard and control possible infectious animal diseases in the Heck cattle population in The Oostvaardersplassen. Report by the Forest Service, The Netherlands, pp. 101 [in Dutch].

Jones, C. 2003. Herpes simplex virus type 1 and bovine herpesvirus 1 latency. Clin. Microbiol. Rev. 16, 79-95.

Kaashoek, M.J., Moerman, A., Madic, J., Rijsewijk, F.A.M., Quak, J., Gielkens, A.L.J., Van Oirschot, J.T., 1994. A conventionally attenuated glycoprotein E-negative strain of bovine herpesvirus type 1 is an efficacious and safe vaccine. Vaccine 12, 439-444.

Kita, J., Anusz, K., 1991. Serologic survey for bovine pathogens in free-ranging European bison from Poland. J. Wildl. Dis. 27, 16-20.

De Koeijer, A., Nodelijk, G., Mars, M.H.., Mourits, M., 2003. Estimation of the reactivation rate of Bovine Herpesvirus 1 from field data. Report by the Animal Sciences Group, Lelystad, The Netherlands, 03/0026609, pp. 20 [in Dutch].

Kramps, J.A., Magdalena, J., Quak, J., Weerdmeester, K., Kaashoek, M.J., Maris-Veldhuis, M.A., Rijsewijk, F.A.M., Keil, G., Van Oirschot, J.T., 1994. A simple, specific, and highly sensitive blocking enzyme-linked immunosorbent assay for detection of antibodies to bovine herpesvirus 1. J. Clin. Microbiol. 32, 2175-2181.

Mars, M.H., 2000. Vaccinology and Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, The Netherlands, pp. 145.

Mollema, L., De Jong, M.C.M., Boven, M., 2005. Prolonged persistence of bovine herpesvirus in small cattle herds: a model based analysis. Epidem. Infect. 133, 137-148.

Nieuwdorp, E., 2000. Hellegatsplaten: Year-round grazing regime with Heck cattle and Fjord horses: report on monitoring research from April 1999 till March 2000. Report by the Forest Service, district Delta Nord, The Netherlands, pp. 27 [in Dutch].

Van Oirschot, J.T., Kaashoek, M.J., Maris-Veldhuis, M.A., Weerdmeester, K., Rijsewijk, F.A.M., 1997. An enzyme-linked immunosorbent assay to detect antibodies against glycoprotein gE of bovine herpesvirus 1 allows differentiation between infected and vaccinated cattle. J. Virol. Methods 67, 23-34.

Platteeuw, M., Cornelissen, Beemster, N., Altenburg, W., Van der Heide, Y., 2000. Vegetation, grazing and birds in a fresh water swamp: monitoring programme 'De Oostvaardersplassen', 1998/1999. In: report by the institute for Inland Water Management and Waste Water Treatment, Lelystad, The Netherlands, 2000.120X, pp. 109 [in Dutch].

Sheffy, B.E., Rodman, S., 1973. Activation of latent infectious bovine rhinotracheitis virus infection. J. Am. Vet. Med. Assoc. 163, 850-851.

Taylor, S.K., Lane, V.M., Hunter, D.L., Eyre, K.G., Kaufman, S., Frye, S., Johnson, M.R., 1997. Serologic survey for infectious pathogens in free-ranging American bison. J. Wildl. Dis. 33, 308-311.

Van den Tempel, R., 1987. The behaviour of the Heck cattle population in Slikken van Flakkee. Report by the Forest Service and the Ministry of Agriculture, Nature and Food Quality, The Netherlands, pp. 99.

Van Tienhoven, J., 2000. Social structure of a Heck cattle population: in Hellegatsplaten. Report by the Dutch Forest Service, district Delta Nord, The Netherlands, pp. 29 [in Dutch].

Vonk Noordegraaf, A. 2002. Simulation modelling to support national policy making in the control of bovine herpesvirus 1. Ph.D. Thesis. University of Wageningen, The Netherlands, pp.159.

Vulink, J.Th., 2001. Hungry Herds: management of temperate lowland wetlands by grazing. Ph.D. Thesis. University of Groningen, The Netherlands, pp. 394.

Van Vuure, C. (ed.), 2005. Retracing the Aurochs: history, morphology and ecology of an extinct wild ox. Pensoft, Sofia-Moscow, pp. 431.

De Wit, J.J., Hage, J.J., Westenbrink, F., 1998. A comparative study of serological tests for use in the bovine herpesvirus 1 eradication programme in The Netherlands. Vet. Microbiol. 61, 153-163.


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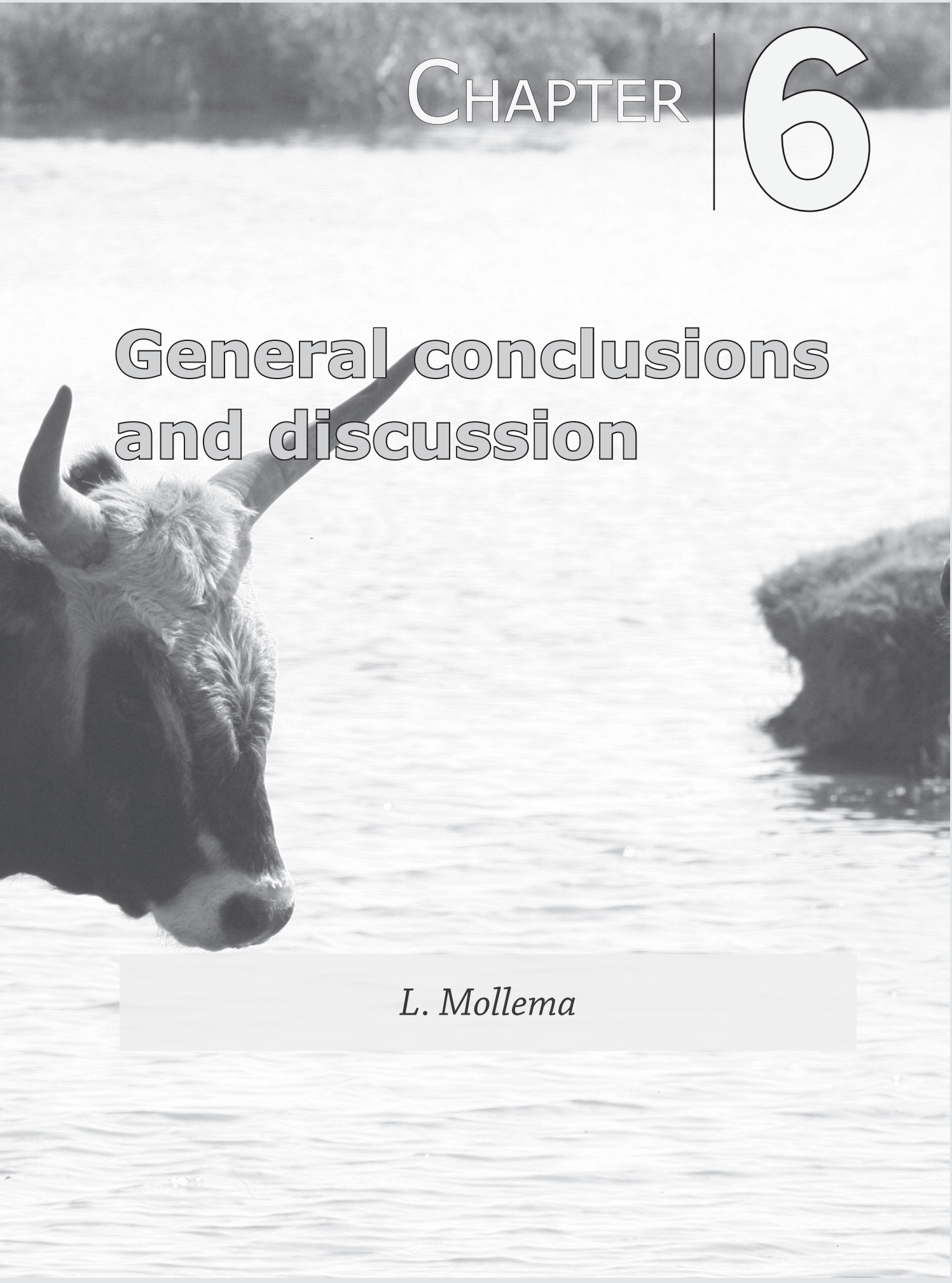


Chapter 6

General conclusions and discussion

L. Mollema

The objective of this thesis was to study the role of feral animals for the possible introduction of infectious pathogens in domestic animals. BHV1 was chosen as an example because farmers currently attempt to eradicate BHV1 in their domestic cattle and thus possible persistence of BHV1 in feral animals is an issue for them. The specific research question became whether BHV1 could persist in feral Heck cattle and red deer populations. Although persistence in feral animal populations does not necessarily imply a risk for domestic animals, it is an important condition for such a risk to occur.

In chapter 2 results are presented from which it is clear that BHV1 may persist in very small cattle populations. For realistic parameter values (reproduction ratio of a single outbreak (R_1) = 3.2; reactivation rate = 0.09 year^{-1}; mortality rate = 0.1 year^{-1}) the mean time to extinction was already in the order of 100 years in a population of 10 animals. With increasing population sizes (e.g. population size = 50) the mean time to extinction increased strongly, and could be quickly in the order of millions of years. The population size of the three Heck cattle populations living in Dutch nature reserves varied between approximately 100 and 600 animals, which would in principal be large enough for BHV1 to persist. However, in the observational study of the contact structure of the eastern subpopulation of Heck cattle in 'The Oostvaardersplassen' (OVP) also smaller subgroups of less than ten animals and solitary animals were observed (chapter 3). The question then was whether the observed contact structure would make persistence of BHV1 in the Heck cattle population less likely. The conclusion that BHV1 may persist in the Heck cattle population in OVP was derived because:

1. The observed seroprevalence of BHV1 in the Heck cattle population in OVP from 1997 till 2003 was on average 89% and did not show a significant decrease (chapter 5).
2. The virus may already persist in one animal for several years and in small groups even longer because a carrier animal may infect other animals in its group. The mean age at infection has been estimated at 1.7 years (chapter 5) and the mean host lifetime is about ten years. Thus on average BHV1 may persist in an individual for about 8 years and during this 8 years the virus may be re-excreted on average about once in one animal (with the reactivation rate given in chapter 2) or once in every five animals (with the reactivation rate given in chapter 5).
3. If a re-excreting animal is able to infect a susceptible animal then it was not taken into account in the calculation of the sizes of groups that, the chain of infections could have continued through the newly infectious animal and thus increasing the infectious period with 14 days. If the infectious period of 14 days applies, then the observations in chapter 2 show that larger groups exist during the same study

periods.

4. Small groups and solitary animals did not exist during the whole year as during summer larger groups were formed.

In addition, it was observed that red deer could be serologically positive for BHV1 (Dutch Animal Health Service, unpublished results) and therefore the role of red deer in the transmission of BHV1 was investigated. In chapter 4 it was found that red deer can be infected with BHV1, but no transmission of BHV1 was observed among red deer. The one-sided 95% confidence interval for R_1 was [0.0 – 0.94]. Based on these results, we would expect only minor outbreaks of BHV1 to occur in red deer populations. Moreover, it was found that the observed seropositivity for BHV1 in red deer in 'the Oostvaardersplassen' could equally well be due to infection with their own herpesvirus, cervid herpesvirus 1. It can be concluded that at most red deer will be a satellite group for BHV1 and therefore do not play a significant role in BHV1 transmission. Consequently, it is not necessary for the eradication of BHV1 in cattle to eradicate BHV1 in red deer populations as well.

Eradication of BHV1 in the Heck cattle populations by means of vaccination was studied in chapters 2 and 5. In chapter 5 the dynamics of BHV1 in a population of hundred animals of which a large part was yearly vaccinated were simulated. From the simulation results it was clear that BHV1 became already extinct within fifteen years in three out of twenty populations. In the other partly vaccinated populations only a small percentage of latently infected animals were left in the population at the end of the simulations. Also from the serological data in the two partly vaccinated Heck cattle populations in 'Slikken van Flakkee' (SFL) and 'Hellegatsplaten' (HPL) it became clear that the seroprevalence for BHV1 decreased due to vaccination. It can be concluded that vaccination of a large part of the Heck cattle population might be an effective tool for BHV1 eradication in at least the smaller Heck cattle populations in SFL and HPL. But, it should be taken in mind that major outbreaks still could take place as the reproduction ratio (R_1) for BHV1 in the partly vaccinated populations is still above one. For example major outbreaks might have occurred in SFL as the seroprevalence in SFL had decreased less compared to HPL, despite vaccination had started earlier in SFL.

Other options for BHV1 eradication in large feral cattle populations

For a large feral cattle population such as the Heck cattle population in OVP, vaccination of the whole population by injecting the vaccine intramuscularly is not feasible. Thus in practice only a small part of the population becomes vaccinated and with the current vaccination strategy this implies that major outbreaks are still possible (chapter 5).

Cohen et al. (2003) proposed a vaccination strategy, which - although only part of the population is vaccinated - is more effective than randomly vaccinating the same proportion of

the population. The strategy is called acquaintance immunisation, and implies immunisation of random acquaintances of random nodes (animals). A random fraction *p* of the *N* nodes was chosen and then it was looked for a random acquaintance with which the nodes were in contact. This strategy requires no knowledge of the node degrees or any other global knowledge, as do targeted immunisation strategies, which we proposed in chapter 2. Cohen et al. (2003) showed that the immunisation threshold dramatically reduced with acquaintance immunization compared to random immunisation.

Regarding the Heck cattle population in OVP this vaccination strategy may be applied for example with an intranasal live BHV1 vaccine, which transmits to some extent to other individuals via the acquaintance network. The idea is that with this vaccination strategy the acquaintances of those individuals with the largest number of acquaintances will all become vaccinated, which will limit the transmission. However, the danger exists that the vaccine strain will persist in the Heck cattle population if the vaccine strain that will be used can also reactivate.

Another possible option to obtain adequate vaccination coverage might be by the use of oral vaccines. However, an oral vaccine for BHV1 is not available at this moment and another problem for the Heck cattle population in OVP is that feeding of the animals is considered an undesirable intervention with the natural developments in the nature reserve.

For BHV1 in domestic cattle, removal of the last latently infected animals is applied in addition to vaccination with a marker vaccine. In the Dutch eradication programme for BHV1 in domesticated cattle removal is advised when the seroprevalence has decreased to less than 10% (Dutch Animal Health Service). However, for the Heck cattle population it will be difficult to find all latently infected individuals. As older animals have a higher probability to be infected with BHV1 it may be decided to remove all the older animals. The danger then exists that not all latently infected animals have been removed in the population, which could result in a major outbreak after reactivation of the virus in the latently infected animal.

Another possible option of establishing a population free of BHV1 is by placing the newborn calves from an existing population in a new area. These calves were then separated from the rest of the population. For existing infected populations such a newly established population free of BHV1 could be used to replace the infected population. However, especially for large populations this is a drastic intervention, which will not be attempted unless there are serious reasons to do so.

Should BHV1 eradication take place anyway?

For an infectious disease that has to be eradicated among domestic animals, the question arises whether feral animals will make the eradication more difficult or even impossible.

Feral animals can only hamper this eradication when firstly the infectious pathogen persists in the feral animal population and secondly transmission occurs between the feral animal population and the domestic animal population. In this thesis we only addressed the first condition: persistence of BHV1 in Heck cattle and red deer.

It can be assumed that concurrent with transmission within the feral Heck cattle population also domestic animal populations are at risk. Only some information on the risks of transmission of BHV1 by different transmission routes between domestic cattle populations is available. The most important risk factors for transmission of BHV1 between domestic cattle farms were determined by Schaik et al. (1998; 2002). These risks are purchase of infectious cattle, returning export cattle (i.e. cattle removed from the farm for sale were allowed to return when not sold) and professional and occasional visitors. Schaik et al. (1998) also showed that a larger distance to the nearest cattle farm was a preventive factor. For the Heck cattle populations discussed in this study, measures were taken to avoid direct contact between feral cattle and domestic cattle. Thus for possible transmission of BHV1 between feral and domestic cattle probably only distance related transmission of BHV1 has to be considered.

Then it only remains to obtain an estimate for the risk at possible distance related transmission. The probability of BHV1 transmission to a nearby domestic cattle herd within a radius of 1 km of a previously certified BHV1-free domestic cattle herd with a confirmed BHV1 outbreak, was calculated to be 5% (Holzhauer et al., not published).

This probability was then extrapolated to a probability of BHV1 transmission to nearby domestic cattle herds from the Heck cattle population in OVP by calculating the mean number of outbreaks per year that might occur in the eastern part of the Heck cattle population in OVP. The probability was estimated at 13%, which means that if eight herds are situated within a radius of 1 km of OVP that each year on average one domestic cattle herd may become infected. However, only one domestic cattle herd lies within a radius of 1 km of OVP (Griekspoor, pers. commun.). For this one domestic cattle herd the result means that this herd may become infected with BHV1 on average once every eight years. The estimated probability has to be interpreted carefully as the actual probability will probably be lower than estimated here because:

1. The disease dynamics of BHV1 in Heck cattle have been assumed to be similar as in domestic cattle but this does not need to be the case. Disease caused by BHV1 has never been reported in Heck cattle whereas it is seen in domestic cattle.
2. The average outbreak size has been estimated to be two or three times smaller in

the Heck cattle population than in the domestic cattle populations with a confirmed BHV1 outbreak described in Holzhauer et al. (Not published).

Eradication of BHV1 in the Heck cattle population would therefore probably not be necessary because it seems that BHV1-infected Heck cattle are no direct threat for the eradication of BHV1 in nearby domestic cattle herds.

Research on the probability of possible transmission of infectious diseases between wildlife and domestic animal populations becomes even more important because of the further development of the National Ecological Network, which is a coherent network of nature areas. The aim of the network is to realise 728,500 hectares of nature by 2018. The National Ecological Network is intended to link up with nature areas in Germany and Belgium in the future, to strengthen the Pan-European Ecological Network (PEEN). Wildlife with their infectious agents may then be able to travel over long distances and congregate with other wildlife species and in addition lead to infection in domestic animals. The various wildlife species, the large populations and the exchange of individuals with other countries may make the eradication of an infectious agent in a population much more difficult compared to only one animal species in one defined area and in one country.

References

Cohen, R., Havlin, S., Ben-Avraham, D. 2003. Efficient immunization strategies for computer networks and populations. Physical review letters 91.

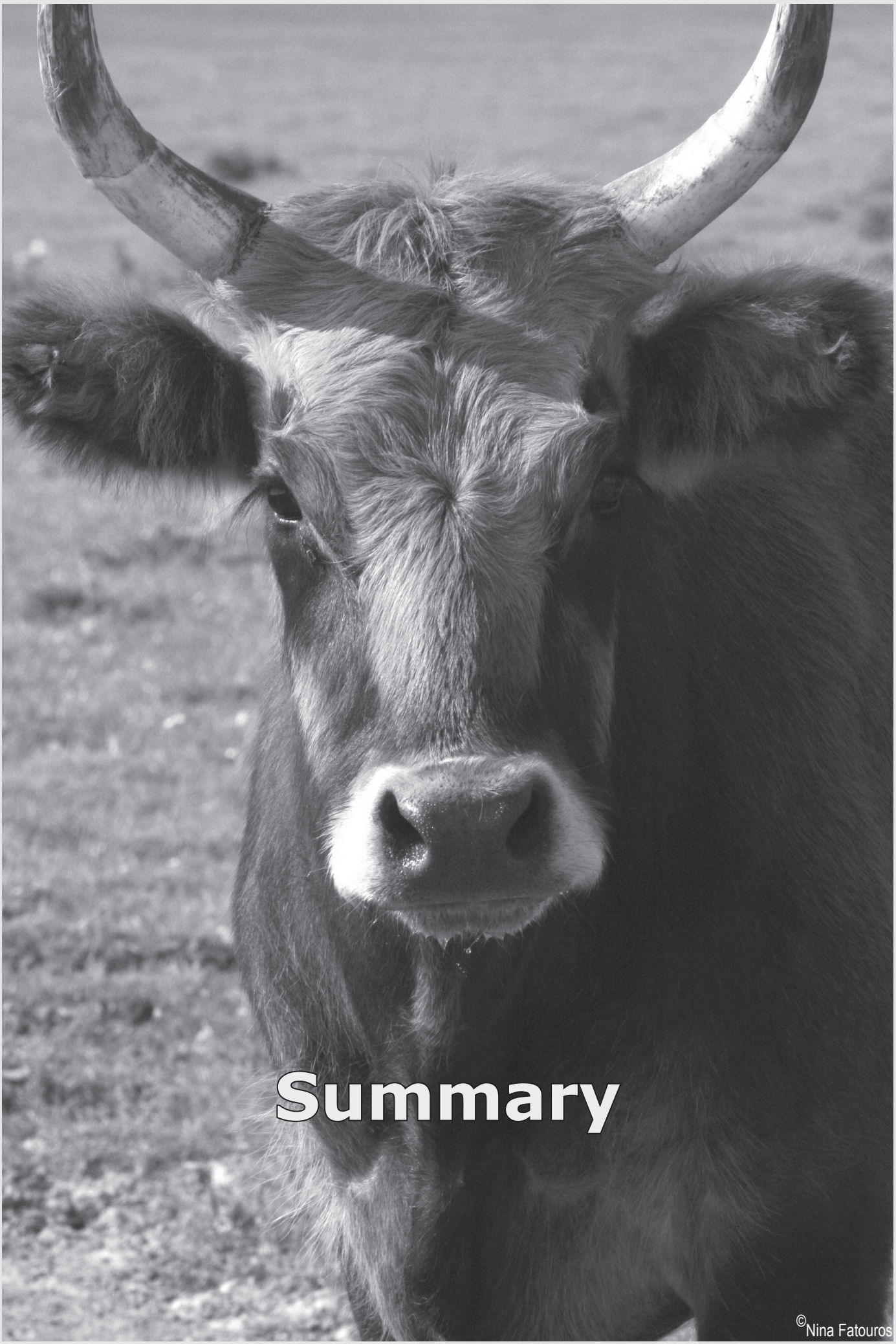
Dutch Animal Health Service (Gezondheidsdienst voor Dieren), Deventer, The Netherlands (http://www.gd-dieren.nl).

Holzhauer, M., De Jong, M.C.M., Hage, J.J. Bovine herpesvirus 1 circulation within and between herds after virus introduction into certified BHV1-free herd. [Not published].

Mars, M.H., 2000. Vaccinology and Epidemiology of bovine herpesvirus 1 infections. Ph.D. Thesis. University of Utrecht, The Netherlands, pp 145.

Van Schaik, G., Dijkhuizen, A.A., Huirne, R.B.M., Schukken, Y.H., Nielen, M., Hage, H.J., 1998. Risk factors for existence of Bovine Herpes Virus 1 antibodies on nonvaccinating Dutch dairy farms. Prev. Vet. Med. 34, 125-136.

Van Schaik, G., Schukken, Y.H., Nielen, M., Dijkhuizen, A.A., Barkema, H.W., Benedictus, G., 2002. Probability of and risk factors for introduction of infectious diseases into Dutch SPF dairy farms: a cohort study. Prev. Vet. Med. 54, 279-289.


Summary
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Introduction

In The Netherlands, cattle farmers have to comply with several European and/or national rules for keeping animals in order to minimise the spread of specific pathogens. For example, cattle farmers have to identify and register their animals, surveillance of their cattle for certain diseases has to take place, and vaccination against specific pathogens has to be implemented. Feral animals in nature reserves may also be susceptible to infection with these pathogens. However, these rules do not have to be implemented in the feral animal populations in nature reserves to the extent as in domestic cattle populations. Conservationists in their strive for self sustaining nature, want to intervene in these feral animal populations as little as possible.

As a result of the less stringent rules for keeping animals in nature reserves as compared to keeping cattle at farms, there is an ongoing debate between farmer organisations, conservationists and government about whether the health status of feral cattle jeopardises the health status of domestic cattle.

In this respect, eradication of bovine herpesvirus 1 (BHV1) is the most prominent acute problem. BHV1 is a herpesvirus and causes infectious bovine rhinotracheitis (IBR) and genital infections. In May 1998 a compulsory programma to eradicate BHV1 had started in domestic cattle. Although this compulsory programme is suspended since February 1999, eradication of BHV1 still takes place on a voluntary basis. As antibodies against BHV1 also have found to be present in various feral animal populations living in nature reserves in The Netherlands, the question arises whether the BHV1-infected feral animal populations are a threat for the eradication of BHV1 in domestic cattle populations. These feral animal populations are: approximately 600 Heck cattle in 'the Oostvaardersplassen' (OVP), approximately 1000 red deer in OVP, approximately 130 Heck cattle in 'Slikken van Flakkee' (SFL) and approximately 139 Heck cattle in 'Hellegatsplaten' (HPL). Heck cattle are a crossbred from various cattle breeds resembling the extinct aurochs *Bos primigenius*. In the animal populations in OVP no eradication of BHV1 takes place, whereas in the Heck cattle populations in SFL and HPL vaccination against BHV1 had started since 1998 and 2000, respectively.

The objective of the research described in this thesis is answering the question whether these above mentioned Heck cattle and red deer populations are a threat for the eradication of BHV1 in domestic cattle. For this purpose, the role of the Heck cattle and the red deer populations in the spread and survival of BHV1 is studied.

BHV1 causes a variety of clinical signs, e.g. rhinotracheitis, fever, conjunctivitis, drop in milk production, abortion, encephalitis, and lesions of the mucous membranes of the genital tract. Morbidity due to BHV1-infection has been reported to range between 20% and 100%. Mortality due to BHV1 has been reported to vary between 1% and 12%.
Before the introduction of the compulsory eradication programme, BHV1 infections in cattle were widespread in The Netherlands. For instance, a BHV1 bulk milk survey in 1994 revealed that at least 84% of the dairy herds had seropositive cattle, while 12% of these herds had seropositive young stock. After the introduction of this programme, the seroprevalence for BHV1 of milking cows in The Netherlands decreased strongly from 40% in 1997 to 22% in 2000. At the same time the total number of BHV1-free certified herds had increased from 3000 herds in 1997 to almost 16,000 herds in 2000.
If a susceptible animal is infected with BHV1, the infectious animal may excrete the virus for approximately 14 days. Once animals are infected with a herpesvirus they remain carriers of the virus for life. A carrier animal is an animal that is already infected with BHV1. Under certain stress conditions the virus can reactivate in the carrier animal after which carrier animals become infectious again. Carrier animals may re-excrete the virus for approximately 5 days, which is shorter than the period a primary infectious animal excretes virus. Reactivation has been observed after stress, transport, super infections with parainfluenza virus type 3 or *Dictyocaulus viviparous*, at parturition, after treatment with dexamethasone and adrenocorticotroop hormoon (ACTH), after uptake of 3-methylindole or by an unknown cause. Carrier animals may establish primary infections in susceptible animals after reactivation of the virus. If not all carrier animals have died or have been taken out of the population before the virus is transmitted, BHV1 may persist in the population.

The role of Heck cattle and red deer in the spread and persistence of BHV1

In chapter 2 of this thesis persistence of BHV1 in cattle populations of various sizes was studied. This was done by estimating the mean time to extinction using a fully stochastic model. The model included for example stochasticity in the size of the outbreak and demographic stochasticity. For realistic parameter values the mean time to extinction was already in the order of 100 years in a population of 10 animals. For a population of 50 animals, the mean time to extinction increased strongly, and could be quickly in the order of millions of years. The impact of stochasticity in the size of the outbreak on the time to extinction was that the time to extinction was substantially larger for values of the reproduction ratio of a single outbreak (R_1) close to one. The impact of demographic stochasticity and for various host lifespans was that for R_1 near to or just above 1 the mean times to extinction were larger. For

larger values of R_1, the mean times to extinction were considerably smaller than when not taking demographic stochasticity into account.

Chapter 3 describes the results of the observations of the contact structure of part of the Heck cattle population in OVP. The contact structure was observed to study whether BHV1 may also persist in the Heck cattle populations. Additionally, the hypothetical effect of the observed contact structure on BHV1 transmission was quantified. The number of different animals with whom an animal had contact, was observed for almost a whole year and five days in the week. Only the contacts with a certain probability of BHV1 transmission (e.g. nose-to-nose contacts) were observed. Further on it will be discussed whether BHV1 can survive in the Heck cattle populations when taking into account the observed contact structure.

Most contacts were observed during summer and fewest contacts during winter-spring. During autumn and winter-spring small groups and solitary animals existed for the short infectious period of five days whereas during summer all individuals were observed together direct of indirect in the same group. For the long infectious period of fourteen days almost all animals were seen together in the same group during all study periods. The hypothetical effect of the contact structure on BHV1 transmission was that the contact structure did differ significantly from random mixing. This type of mixing is predominantly used in epidemiological models. The variation in the number of contacts was higher than under random mixing, which meant that the virus might spread to a larger extent than under random mixing assumptions.

After studying the persistence of BHV1 in the Heck cattle populations, the effects of vaccination were studied on the dynamics and persistence of BHV1 in the Heck cattle populations (chapter 5). For this purpose, serological data of BHV1 in the Heck cattle populations were combined with model simulations of the dynamics of a BHV1 infection. The average observed seroprevalence for BHV1 in respectively OVP, SFL and HPL was 89%, 59% and 49%. The seroprevalence stayed relatively high in OVP, whereas it decreased in the two other populations due to vaccination. Despite that not all animals were vaccinated, BHV1 became extinct in three out of twenty simulated populations within fifteen years. It should be mentioned that major outbreaks still could take place in the partly vaccinated Heck cattle populations as the reproduction ratio was not below one. In that case the time to extinction would still take a long time, probably longer than desirable for eradication purposes.

In chapter 4 the extent to which BHV1 may spread among red deer was quantified by performing two transmission experiments. The results of these experiments showed that red deer can be infected with BHV1 and excrete BHV1 virus. But, no transmission of BHV1 was observed among red deer. Further it was demonstrated that the observed seropositivity for

BHV1 in red deer in OVP could equally well be due to infection with their own herpesvirus, cervid herpesvirus 1.

Conclusions and Discussion

One of the objectives of this thesis was to study whether BHV1 may persist in the Heck cattle populations taking into account the observed contact structure. The conclusion that BHV1 may persist in the Heck cattle populations without vaccination was derived because:

1. The observed seroprevalence of BHV1 in the Heck cattle population in OVP from 1997 till 2003 was on average 89% and did not show a significant decrease (chapter 5).
2. The virus may already persist in one animal for several years and in small groups even longer because a carrier animal may infect other animals in its group.
3. If a re-excreting animal is able to infect a susceptible animal then it was not taken into account in the calculation of the sizes of groups, that the chain of infection could have continued through the newly infectious animal and thus increasing the infectious period with 14 days. If the infectious period of 14 days applies, then the observations in chapter 2 show that larger groups exist during the same study periods.
4. Small groups and solitary animals did not exist during the whole year as during summer larger groups were formed compared to the other study periods (chapter 2).

Vaccination of a large part of the Heck cattle population might be an effective tool for BHV1 eradication in at least the smaller Heck cattle populations in SFL and in HPL. In 2005 the percentage of BHV1 seropositive Heck cattle in HPL had decreased to 11% and in SFL to 35%, whereas in 2004 the percentage of seropositive Heck cattle was respectively 20% and 43%. Serological data from the Heck cattle population in HPL showed that this year (2006) the percentage of BHV1 seropositive cattle had decreased to 8%. If no eradication measures for BHV1 are taken than BHV1 will persist in the Heck cattle populations.

From the results described in chapter 4 it can be concluded that red deer alone do not play a significant role in BHV1 transmission. Consequently, for the eradication of BHV1 in domestic cattle, eradication of BHV1 does only have to take place in the Heck cattle populations. But, should eradication of BHV1 really have to take place in the Heck cattle population? Eradication of BHV1 should only have to take place, if the infected Heck cattle population is a threat for the eradication of BHV1 in domestic cattle populations. Therefore, we have to know if transmission of BHV1 occurs from the Heck cattle population to the domestic cattle populations. The result of the calculation in chapter 5 showed that several BHV1 outbreaks might yearly take place in the Heck cattle population in OVP. Concurrent with transmission within the Heck cattle population also domestic cattle populations may be at risk. The

probability of BHV1 transmission to a nearby domestic cattle herd within a radius of 1 km of a previously certified BHV1-free domestic cattle herd with a confirmed BHV1 outbreak, was already calculated to be 5%. This probability was then extrapolated to a probability of BHV1 transmission to nearby domestic cattle herds from the Heck cattle population by taking into account the mean number of outbreaks per year that might occur in OVP. The probability was estimated at 13%, which means that if eight herds are situated within a radius of 1 km of OVP that each year on average one domestic cattle herd may become infected. However, only one domestic cattle herd lies within a radius of 1 km of OVP. For this one domestic herd the result means that this herd may become infected with BHV1 on average once every eight years.

The estimated probability has to be interpreted carefully as the actual probability will probably be lower than estimated here because:

1. The disease dynamics of BHV1 in Heck cattle have been assumed to be similar as in domestic cattle but this does not need to be the case. Disease caused by BHV1 has never been reported in Heck cattle whereas it is seen in domestic cattle.
2. The average outbreak size has been estimated to be two or three times smaller in the Heck cattle population than in the domestic cattle populations with a confirmed BHV1 outbreak.

Eradication of BHV1 in the Heck cattle population would therefore probably not be necessary because it seems that BHV-1 infected Heck cattle are no direct threat for the eradication of BHV1 in nearby domestic cattle herds.

In summary the main conclusions are:

- BHV1 can persist in small cattle populations (e.g. on average 100 years in a population of 10 animals) (chapter 2)
- Minor outbreaks have to be taken into account in the calculation of the mean time to extinction of BHV1 for values of R_1 close to 1. This increases the mean time to extinction (chapter 2)
- Demographic stochasticity has to be taken into account in the calculation of the mean time to extinction of BHV1, especially for large values of R_1 and large host lifespans. This decreases the mean time to extinction (chapter 2)
- Transmission is favoured most during summer and least during winter-spring based on the number of observed contacts (chapter 3)
- The contact structure of the Heck cattle population in OVP does differ significantly from random mixing. The variation in the number of contacts is higher than under random mixing (chapter 3)
- The observed seroprevalence stays relatively high in OVP whereas it decreases in the two other populations due to vaccination (chapter 5)
- BHV1 may become extinct within 15 years in partly vaccinated populations (chapter 5)
- Major outbreaks still can take place in the partly vaccinated Heck cattle populations, on average once per 21 years (chapter 5)
- Red deer can be infected with BHV1 and can excrete BHV1, but no transmission of BHV1 is observed among red deer (chapter 4)

Samenvatting

Deze samenvatting is een vereenvoudigde bewerking van de Engelse, wetenschappelijke samenvatting (Summary).

Inleiding

In Nederland hebben veehouders met verschillende Europese en nationale regels voor het houden van dieren te maken, onder andere om verspreiding van specifieke ziekteverwekkers tegen te gaan. Zo moeten veehouders bijvoorbeeld al hun dieren identificeren en registreren, worden de dieren gecontroleerd op dierziekten en wordt er gevaccineerd tegen bepaalde ziekteverwekkers. Dieren in natuurgebieden kunnen net als dieren op veebedrijven ook vatbaar zijn voor een besmetting met deze ziekteverwekkers. Echter de regels waar de veehouders mee te maken hebben hoeven niet in dezelfde mate te worden toegepast door natuurbeheerders. De reden hiervan is dat dieren in natuurgebieden een ander doel dienen dan dieren op veebedrijven. Omdat de regels minder streng zijn voor het houden van dieren in natuurgebieden is er continue een debat gaande tussen veehoudersorganisaties, natuurbeheerders en het Ministerie van Landbouw over of de gezondheid van dieren in natuurgebieden de gezondheid van dieren op boerderijen bedreigd.
Een voorbeeld van zo'n discussie is het uitroeien van bovine herpesvirus type 1 (BHV1). BHV1 is een herpesvirus en veroorzaakt onder andere infectieuze bovine rhinotracheïtis (IBR), ook wel koeiengriep genoemd. Veehouders willen dit virus uitroeien in hun koeien. Echter verschillende dierpopulaties in natuurgebieden in Nederland blijken ook met dit virus besmet te zijn. Daarom is men bang dat deze dieren mogelijk een bedreiging vormen voor het uitroeien van BHV1 in koeien op veebedrijven, hierna ook wel gehouden koeien genoemd.
In mei 1998 is er een landelijk verplicht uitroeiingsprogramma voor BHV1 gestart in gehouden koeien. Dit uitroeiingsprogramma is voornamelijk ingevoerd om een sterkere handelspositie binnen Europa te verkrijgen en omdat de ziekte hoge kosten met zich meebrengt. Hoewel het verplichte uitroeiingsprogramma sinds februari 1999 geschorst is vanwege een besmette partij met IBR vaccins, vindt uitroeiing van BHV1 in de gehouden koeienpopulaties nog steeds op vrijwillige basis plaats.
De met BHV1-besmette dierpopulaties in natuurgebieden in Nederland waar het in dit proefschrift om gaat zijn: ongeveer 600 Heckrunderen in 'De Oostvaardersplassen' (OVP), ongeveer 1000 edelherten in OVP, ongeveer 130 Heckrunderen in 'Slikken van Flakkee' (SFL) en ongeveer 139 Heckrunderen in 'Hellegatsplaten' (HPL). Heckrunderen zijn een soort 'oerossen' en zijn het resultaat van jarenlang kruisen met verschillende koeienrassen. In de dierpopulaties in OVP worden geen maatregelen toegepast om BHV1 uit te roeien, echter

in de Heckrunderen in SFL en HPL wordt er sinds respectievelijk 1998 en 2000 gevaccineerd tegen BHV1.
Het doel van het onderzoek in dit proefschrift beschreven is het beantwoorden van de vraag of deze hierboven beschreven Heckrunderen en edelherten een bedreiging vormen voor het uitroeien van BHV1 in gehouden koeien. Hiervoor onderzoeken we de rol van de Heckrunderen en edelherten in de verspreiding en overleving van BHV1.

BHV1 kan verschillende ziekteverschijnselen veroorzaken zoals snotteren en/of snurken, neusuitvloeiing in de vorm van slijmerig snot, ooguitvloeiing, koorts, verminderde eetlust, daling in melkproductie, roodheid en beschadigingen van de neusslijmvliezen en afstoten van een kalf. Ziekte als gevolg van een BHV1 besmetting varieert tussen 20% en 100%. De sterfte door BHV1 kan variëren tussen de 1% en 12%.
Vóór de introductie van het verplichte uitroeiingsprogramma van BHV1 kwamen BHV1 besmettingen overal in Nederland voor. Zo liet een melktankonderzoek in 1994 zien dat tenminste 84% van de melkveebedrijven besmette koeien met BHV1 had en gemiddeld 12% van deze bedrijven besmet jongvee had. Ná invoering van het verplichte uitroeiingsprogramma van BHV1 nam het aantal met BHV1 besmette melkkoeien in Nederland sterk af van 40% in 1997 naar 22% in 2000. Op hetzelfde moment nam het aantal officieel BHV1-vrij verklaarde bedrijven toe van 3000 bedrijven in 1997 naar bijna 16000 bedrijven in 2000.
Als een dier besmet is met BHV1, scheidt het besmette dier virus uit voor ongeveer 14 dagen. Dieren die eenmaal besmet zijn met het herpesvirus blijven hun hele leven lang dragers van het virus. Een drager is dus een dier dat al besmet is met BHV1. Het virus kan daarna weer actief worden (reactiveren) na een periode van weerstandsvermindering bijvoorbeeld na transport, ziekte of afkalven. Hierdoor kunnen dragers weer opnieuw virus gaan uitscheiden voor een kortere periode van ongeveer 5 dagen, waarmee ze vatbare dieren kunnen besmetten die het virus dan weer voor ongeveer 14 dagen kunnen uitscheiden. Als niet alle dragers sterven of uit de populatie worden verwijderd voordat het virus is verspreid in de populatie, kan het virus een heel lange tijd overleven in de populatie.

De rol van Heckrund- en edelhertpopulaties in de verspreiding en overleving van BHV1

Hoofdstuk 2 beschrijft het overleven van BHV1 in koeienpopulaties. Hiervoor is een model gemaakt waarmee de gemiddelde overlevingsduur van het virus in koeienpopulaties met verschillende aantallen dieren berekend kan worden. Voor realistische waarden van de

variabelen in het model was de gemiddelde overlevingsduur van BHV1 in een populatie van 10 dieren al 100 jaar. In grotere groepen van bijvoorbeeld 50 dieren nam de gemiddelde overlevingsduur sterk toe en was al snel in de orde van een miljoen jaar.
Hoofdstuk 3 beschrijft de observaties van de contactstructuur van een deel van de Heckrundpopulatie in OVP. Ongeveer een jaar lang vijf dagen in de week is er gekeken naar met hoeveel verschillende runderen een rund per 20 minuten contact had. Alleen contacten met een bepaalde kans op overdracht van BHV1 (bijvoorbeeld neus-neus contact) werden meegenomen in de observaties. Het observeren van contacten is onder andere gedaan om te onderzoeken of BHV1 ook kan overleven in de Heckrundpopulatie.
De meeste contacten werden waargenomen in de zomerperiode en de minste in de winter-lenteperiode. Daarnaast werden in de zomerperiode de meeste dieren samen in één groep gezien. Hierbij maakte het niet uit voor welke tijdsperiode geobserveerd werd. Gedurende de herfst- en winter-lenteperiodes werden er kleine groepen en geïsoleerde dieren in een tijdsbestek van 5 dagen gezien. In de zomerperiode zou dus de meeste verspreiding van BHV1 kunnen plaatsvinden. In de herfst- en winter-lenteperiodes is het mogelijk dat een drager geen contact heeft met een vatbaar dier gedurende de periode van virusuitscheiding waardoor het virus niet kan verspreiden. Verderop bediscussiëren we of BHV1 kan overleven in de Heckrundpopulaties gezien de geobserveerde contactstructuur.
Naast het onderzoek of BHV1 kan overleven in de Heckrundpopulaties, is het effect van vaccinatie op de verspreiding en overleving van BHV1 onderzocht (hoofdstuk 5). Hiervoor werden de data over de BHV1 besmetting in de Heckrundpopulaties gecombineerd met modelsimulaties. Het gemiddelde percentage met BHV1 besmette dieren in respectievelijk OVP, SFL and HPL was 89%, 59% en 49%. Het percentage met BHV1 besmette dieren bleef relatief hoog in OVP, terwijl het door vaccinatie afnam in de andere twee populaties. Hoewel niet alle dieren gevaccineerd waren, stierf het virus binnen 15 jaar uit in drie van de twintig gesimuleerde populaties. Er moet wel rekening mee gehouden worden dat grote uitbraken van het virus nog steeds kunnen plaatsvinden in gevaccineerde Heckrundpopulaties. Dit komt omdat het vaccin de verspreiding van het virus niet voldoende kan verminderen zodat de reproductie ratio (R) < 1 is. R is een getal voor het aantal nieuw besmette dieren veroorzaakt door één ander besmet dier. $R > 1$ betekent dat het virus zich kan verspreiden in de populatie en $R < 1$ betekent dat het virus zich niet of nauwelijks kan verspreiden. Of er grote uitbraken plaats zullen vinden hangt af van of er voldoende vatbare dieren aanwezig zijn in de populatie.
In hoofdstuk 4 is de mate waarin BHV1 kan verspreiden in een groep edelherten onderzocht. Hiervoor hebben we twee transmissie-experimenten uitgevoerd. In het kort, werd in beide experimenten de helft van de dieren besmet met BHV1 en na ongeveer 24 uur werd de

andere helft van de dieren bij de BHV1-besmette dieren geplaatst. Vervolgens is er gekeken naar hoe lang en hoeveel de besmette dieren virus uitscheiden en of de vatbare dieren besmet werden door de BHV1-besmette dieren. Uit onze resultaten bleek dat edelherten besmet kunnen worden met BHV1 en dat ze virus uitscheiden, maar er is geen verspreiding van BHV1 aangetoond onder edelherten.

Conclusies en discussie

Een van de doelen in dit proefschrift was om te onderzoeken of BHV1 kan overleven in de Heckrundpopulaties. Om de volgende redenen kan geconcludeerd worden dat BHV1 kan overleven in de Heckrundpopulaties indien er niet gevaccineerd wordt:

1. Het percentage met BHV1 besmette dieren in de Heckrundpopulatie in OVP van 1997 tot en met 2003 was gemiddeld 89% en liet geen sterke daling zien (hoofdstuk 5).
2. Het BHV1 virus kan enkele jaren overleven in één dier en in kleine groepjes van dieren zelfs langer omdat een drager mogelijk andere dieren in een groep kan besmetten.
3. Een drager van het virus is in staat om een vatbaar dier te besmetten. In de berekening voor de grootte van de groepen is niet meegenomen dat de keten van besmettingen verder zou kunnen verlopen via het nieuw besmette dier. De periode van virusuitscheiding zou daarmee verlengd kunnen worden met 14 dagen. Als de besmettelijke periode 14 dagen is, dan laten de observaties in hoofdstuk 2 zien dat er grotere groepen bestaan in diezelfde studieperiodes.
4. Kleine groepen en geïsoleerde dieren zijn niet het gehele jaar gezien. Gedurende de zomerperiode zijn grotere groepen geobserveerd in vergelijking met de andere studieperiodes.

Vaccinatie van een groot deel van de Heckrundpopulatie blijkt een effectief middel te zijn voor het uitroeien van BHV1 in deze populaties. Dat geldt tenminste voor de kleinere Heckrundpopulaties zoals in SFL en HPL. In 2005 was het percentage met BHV1 besmette dieren in HPL gedaald tot 11% en in SFL tot 35% terwijl het jaar daarvoor het percentage besmette dieren nog respectievelijk 20% en 43% was. Als er geen uitroeiingstrategieën voor BHV1, zoals vaccinatie, worden genomen dan zal BHV1 overleven in de Heckrundpopulaties.

In het onderzoek dat in hoofdstuk 4 is beschreven, is gebleken dat edelherten alleen, geen belangrijke rol spelen in BHV1 verspreiding omdat er geen verspreiding van BHV1 onder edelherten is aangetoond. Daarom hoeft voor het uitroeien van BHV1 in gehouden koeienpopulaties, BHV1 in principe alleen uitgeroeid te worden in de Heckrundpopulaties. Maar de vraag blijft of BHV1 uitgeroeid moet worden in de Heckrundpopulaties. Uitroeiing van BHV1 in de Heckrundpopulaties zou alleen plaats hoeven te vinden indien de besmette

Heckrundpopulaties een bedreiging vormen voor het uitroeien van BHV1 in de gehouden koeienpopulaties. De vraag die dan beantwoord moet worden is of er verspreiding plaatsvindt van BHV1 in de Heckrundpopulatie naar de gehouden koeienpopulaties.

De berekening in hoofdstuk 5 laat zien dat jaarlijks enkele uitbraken van BHV1 kunnen optreden in de Heckrundpopulatie in OVP. Tegelijkertijd met de verspreiding van BHV1 in de Heckrundpopulatie is er een risico op verspreiding van BHV1 naar in de buurt liggende koeienbedrijven. Voor officieel BHV1-vrij verklaarde koeienbedrijven met een bevestigde BHV1 uitbraak, is het risico op verspreiding naar andere koeienbedrijven binnen een afstand van 1 km geschat op 5%. Hieruit is vervolgens een kans geschat op verspreiding van BHV1 naar in de buurt liggende koeienbedrijven vanuit de Heckrundpopulatie in OVP. Dit is gedaan door het gemiddelde aantal uitbraken per jaar dat in de Heckrundpopulatie in OVP zou kunnen plaatsvinden mee te nemen in de berekening. Deze kans werd geschat op 13%. Dit betekent dat als er acht koeienbedrijven in de buurt van de OVP liggen binnen een straal 1 km dat dan elk jaar gemiddeld één bedrijf besmet kan worden. Echter, er ligt slechts één koeienbedrijf op een afstand binnen 1 km van OVP. Voor dit ene bedrijf zou dit resultaat betekenen dat het gemiddeld één keer in de acht jaar besmet zou kunnen worden met BHV1.

De geschatte kans op verspreiding van BHV1 vanuit OVP moet zorgvuldig geïnterpreteerd worden omdat de werkelijke kans waarschijnlijk lager is vanwege de volgende redenen:

1. Er aangenomen is dat het verloop van een BHV1-infectie in Heckrunderen gelijk is aan het verloop van een BHV1-infectie in gehouden koeien. Ziekte veroorzaakt door BHV1 is echter nooit gerapporteerd in Heckrunderen terwijl dit wel het geval is voor gedomesticeerde koeien.
2. De gemiddelde uitbraakgrootte in de Heckrundpopulatie twee of drie keer kleiner is geschat dan in de gehouden koeienpopulaties met een bevestigde uitbraak van BHV1.

Uitroeiing van BHV1 in de Heckrundpopulatie is daarom mogelijk niet nodig omdat het lijkt dat BHV1-besmette Heckrunderen geen directe bedreiging vormen voor het uitroeien van BHV1 op omliggende koeienbedrijven.

Samenvattend zijn de belangrijkste conclusies van dit proefschrift:

- BHV1 kan overleven in kleine koeienpopulaties (bijvoorbeeld gemiddeld 100 jaar in een populatie van 10 dieren) (hoofdstuk 2)
- In de zomerperiode zou de meeste verspreiding van BHV1 kunnen plaatsvinden en het minste in de winter-lenteperiode (hoofdstuk 3)
- Het geobserveerde gemiddelde aantal met BHV1 besmette dieren bleef hoog in OVP, terwijl het door vaccinatie afnam in SFL and HPL (hoofdstuk 5)
- BHV1 kan binnen 15 jaar uitsterven in gedeeltelijk gevaccineerde populaties (hoofdstuk 5)
- Grote uitbraken van BHV1 kunnen nog steeds plaatsvinden in de gedeeltelijk gevaccineerde Heckrundpopulaties, gemiddeld eens in de 21 jaar (hoofdstuk 5)
- Edelherten kunnen besmet worden met BHV1 en BHV1 uitscheiden, maar er is geen verspreiding van BHV1 onder edelherten geobserveerd (hoofdstuk 4)

List of publications

Refereed scientific papers

Peter van Bodegom, Fons Stams, Liesbeth Mollema, Sara Boeke and Peter Leffelaar. 2001. Methane oxidation and the competition for oxygen in the rice rhizosphere. Applied and Environmental Microbiology, 67: 3586-3597.

Mollema, L., De Jong, M.C.M., Van Boven, M. 2005. Prolonged persistence of bovine herpesvirus in small cattle herds: a model based analysis. Epidemiol. Infect. 133, 137-148.

Mollema, L., Rijsewijk, F.A.M., Nodelijk, G., De Jong, M.C.M. 2005. Quantification of the transmission of bovine herpesvirus 1 among red deer (*Cervus Elaphus*) under experimental conditions. Vet. Microbiol. 111, 25-34.

Mollema, L., Koene, P., De Jong, M.C.M. Quantification of the contact structure in a feral cattle population and its hypothetical effect on the transmission of bovine herpesvirus 1. *Accepted*.

Mollema, L., Snoep, J., De Jong, M.C.M. Dynamics and control of bovine herpesvirus 1 in three feral Heck cattle populations: simulations and serological data. *Submitted*.

Other

Lambers, J., Stavast, F., Mollema, L., Nieuwenhuis, M., Van Klink, E. 2005. Opties voor de bestrijding van KVP, MKZ, AVP en SVD bij een uitbraak onder wild in Nederland. Directie Kennis, Ministerie van Landbouw, Natuur en Voedselkwaliteit, Rapport DK nr. 2005/Dk027.

Conference abstracts

Mollema, E., De Jong, M.C.M., Koene, P. 2002. Quantifying the contact patterns of a Heck cattle population in the Netherlands with regard to transmission of bovine herpesvirus 1 (BHV). Proceedings of the 36th International Congress of the International Society for Applied Ethology (ISAE) in Egmond aan Zee, The Netherlands.

Mollema, L., Koene, P., De Jong, M.C.M. 2003. Determining who has contact with whom for a feral cattle population with regard to transmission of a respiratory infection. Proceedings of the 10th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), Viña del Mar, Chili.

Curriculum vitae

Elisabeth Mollema (Liesbeth) werd op 7 mei 1976 geboren te Franeker. Na het behalen van haar VWO-diploma in 1994 aan het Andreas College in Drachten, verruilde zij de provincie Friesland voor de provincie Gelderland om te beginnen met de studie biologie aan de Landbouwuniversiteit Wageningen (nu Wageningen Universiteit). Zij koos vervolgens voor de richting mathematische theoretische biologie binnen de studie biologie. Haar eerste afstudeervak deed ze bij de vakgroep Theoretische Productie Ecologie. Zij bestudeerde daar de invloed van methanotrofen op de reductie van methaan emissie in rijst aan de hand van een model en experimenten. Vervolgens ging ze samen met Tessa Pronk naar Israël voor een afstudeervak en woonde ze voor een halfjaar in een kibboets in En Afeq. In het natuurreservaat in En Afeq werd de invloed van begrazing op de vegetatie samenstelling onderzocht. Haar laatste afstudeervak was bij de vakgroep Wiskunde en ging over de dynamica van het 'African cassava mosaic virus' in een cassave gewas. In september 1999 studeerde zij af en in januari 2000 begon ze met het pendelen tussen Wageningen en Lelystad. In Lelystad begon ze met het in dit proefschrift beschreven promotieonderzoek. Zij was hiervoor aangesteld bij de leerstoelgroep Kwantitatieve Veterinaire Epidemiologie van Wageningen Universiteit en gedetacheerd bij de gelijknamige groep aan het instituut voor Dierhouderij en Diergezondheid, ID-Lelystad (nu Animal Sciences Group van Wageningen UR). Sinds juli 2005 werkt zij als onderzoeker epidemioloog bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in Bilthoven. Zij werkt daar onder andere mee aan een groot landelijk onderzoek naar de bescherming van de Nederlandse bevolking tegen infectieziekten waartegen gevaccineerd wordt in het Rijksvaccinatieprogramma. Zij woont nog steeds in het gezellige Wageningen.

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Paul, jij bent de hele aio-periode erbij geweest. Vooral bij het contactonderzoek in de Oostvaardersplassen (OVP) was je nauw betrokken. Ik heb veel van je geleerd over gedragsonderzoek, bedankt daarvoor. Daarnaast ben je ook een prettig persoon om mee te werken en dat is natuurlijk fijn als je samen hele dagen in de OVP doorbrengt.

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Liesbeth

Training and Supervision Plan by Graduate School WIAS

EDUCATION AND TRAINING

The Basic Package	year	cp*
Course on philosophy of science and/or ethics	2000	
Wias common course	2001	
Subtotal Basic Package		**3**
Scientific Exposure		
International conferences		
Mathematical Modeling of Population dynamics (MMPD), Bedlowa, Polen	2002	
International congress of the International Society for Applied Ethology (ISAE), Egmond aan Zee, The Netherlands	2002	
International Symposium on Veterinary Epidemiology and Economics (ISVEE), Vina del Mar, Chile	2003	
Seminars and workshops		
The genetics of resistance to infectious diseases, Wageningen	2000	
The ecology of disease virulence, Wageningen	2000	
WIAS Science Day, Wageningen	’00-’04	
Dutch Society for Veterinary Epidemiology and Economics (VEEC), Utrecht, Wageningen, Leuven (België)	’00-‘01 /’03	
BSE-congres, Ede	2001	
Dutch Society for the Health of Wildlife, Amsterdam	2002	
Presentations		
WIAS Science Day (poster)	2002	
Symposium over gezondheidsaspecten van grote grazers in natuurterreinen (oral)	2002	
International Congress of the International Society for Applied Ethology (oral)	2002	
Mathematical Modeling of Population dynamics (oral)	2002	
International Symposium on Veterinary Epidemiology and Economics (oral)	2003	
WIAS Science Day (oral)	2004	
Subtotal International Exposure		**14**

In-Depth Studies	year	cp*
Disciplinary and interdisciplinary courses		
Mathematical epidemiology of infectious diseases, Utrecht	2000	
Winter school on population dynamics, Woudschoten	'00/'02	
Summer school of the European Society for Mathematical and Theoretical Biology (ESMTB), Martina Franca, Italy	2000	
3rd International summer school on infectious disease epidemiology, University of Bielefeld, Germany	2001	
AIO Seminar course infection & immunity, Bilthoven	2001	
Summer school on Wildlife health, Ameland	2003	
PhD students' discussion groups		
Biology underpinning animal sciences: broaden your HORIZON, WIAS	2001	
Subtotal In-Depth Studies		**12**
Professional Skills Support Courses		
Algemene inleiding Mathematica, CANdiensten, Amsterdam	2000	
ID-Lelystad Course Techniques for scientific writing, Lelystad	2002	
WIAS Course on techniques for writing and presenting a scientific paper, Wageningen	2002	
Career orientation for PhD students, Wageningen	2004	
Subtotal Professional Skills Support Courses		**4**
Didactic Skills Training		
Lecturing		
Course 'Hoorcollege geven', Onderwijsondersteuning, Wageningen	2001	
Supervising theses		
Supervising three MSc major students	'02/'03	
Subtotal Didactic Skills Training		**7**
Management Skills Training		
Organisation of seminars and course:		
WIAS Science Day	2002	
Membership of boards and committees:		
Member of PhD board (WIAS)	2002	
Subtotal Management Skills Training		**4**
Education and Training Total		**44**

* One credit point (cp) equals a study load of approximately 28 hours

Notes

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