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A. Meyer,[1,*] L. Zamir,[2] A. Ben Yair Gilboa,[2] B. Gelman,[3] D. U. Pfeiffer,[1,4] and T. Vergne[1,5]

Various foot-and-mouth disease (FMD) virus strains circulate in the Middle East, causing frequent episodes of FMD outbreaks among Israeli livestock. Since the virus is highly resistant in semen, artificial insemination with contaminated bull semen may lead to the infection of the receiver cow. As a non-FMD-free country with vaccination, Israel is currently engaged in trading bull semen only with countries of the same status. The purpose of this study was to assess the risk of release of FMD virus through export of bull semen in order to estimate the risk for FMD-free countries considering purchasing Israeli bull semen. A stochastic risk assessment model was used to estimate this risk, defined as the annual likelihood of exporting at least one ejaculate of bull semen contaminated with viable FMD virus. A total of 45 scenarios were assessed to account for uncertainty and variability around specific parameter estimates and to evaluate the effect of various mitigation measures, such as performing a pre-export test on semen ejaculates. Under the most plausible scenario, the annual likelihood of exporting bull semen contaminated with FMD virus had a median of $1.3 * 10^{-7}$ for an export of 100 ejaculates per year. This corresponds to one infected ejaculate exported every 7 million years. Under the worst-case scenario, the median of the risk rose to $7.9 * 10^{-5}$, which is equivalent to the export of one infected ejaculate every 12,000 years. Sensitivity analysis indicated that the most influential parameter is the probability of viral excretion in infected bulls.

KEY WORDS: Bull semen; export risk assessment; foot-and-mouth disease; Israel

1. INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most contagious animal diseases. It affects all cloven-hoofed animals such as cattle, pigs, and sheep. Although the mortality rate is generally low, the morbidity rate can be very high in naïve populations.[1] In acutely infected animals, FMD virus can be found in all secretions and excretions including expired air, saliva, skin lesions, urine, feces, and semen. The two main transmission routes involve contact between susceptible and infectious animals or contaminated fomites such as vehicles, feed, and humans.[2] Secondary routes of infection involve the consumption of contaminated meat products (mainly swill feeding in pigs), consumption of contaminated milk by the newborn, artificial insemination with contaminated semen, and inhalation of infectious aerosols (airborne transmission may occur under favorable climatic conditions over more than 100 kilometers).[3]

[1]Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College, London, UK.
[2]Israel Veterinary Services, Beit-Dagan, Israel.
[3]Kimron Laboratory Institute, Israel Veterinary Services, Beit-Dagan, Israel.
[4]School of Veterinary Medicine, City University of Hong Kong, Hong Kong SAR.
[5]MIVEGEC Group (UMR CNRS/IRD/UM2), Montpellier, France.
*Address correspondence to Anne Meyer, Royal Veterinary College, Hawkshead Lane, Hatfield AL97TA, UK; annemeyer@live.fr.

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Various FMD virus strains circulate in the Middle East and FMD outbreaks occur almost every year in the area, causing large economic losses in these countries[4] in spite of a regional FMD control program in place involving Egypt, Israel, Jordan, and the Palestinian Authority.[5] The vaccination of high-producing cattle such as dairy herds limits the losses due to the circulation of the virus;[6] however, it was reported that only Israel implements a "successful consistent vaccination policy"[3] in the Middle East. Over the last 10 years, all outbreak strains isolated in Israel were identified as serotype O, with the exception of a serotype A outbreak in 2009.[7]

Israel is involved in international trade of bull semen with other non-FMD-free countries. The production of Israeli bull semen doses for export is handled by a cooperative business, whose bull stud and insemination center is located in the Kanot Veterinary District. The facility houses an average of 180 Israeli Holstein bulls selected based on their genetic potential. Calves are chosen around the age of 10 days in dairy farms and raised within the cooperative's facilities until they are transferred to the bull stud and insemination center. Although no FMD outbreaks have occurred in the bull stud since 1950, the possibility of an outbreak in the future cannot be excluded. The closest outbreak in the last 10 years occurred in 2007 in a mixed cattle farm located 15 km away, which suggests that the virus may circulate in the area. High biosecurity measures are in place to protect the bulls' health, including confinement of the bulls within an access-controlled stud and annual FMD vaccination. In Israeli cattle farms, the time between the onset of the clinical signs to the suspicion of an FMD outbreak (i.e., time before detection) is highly variable, from 3 days for the index case of the 2006 epidemic to 13 days in 2007 (Israeli Veterinary Services (IVS), personal communication). However, it is expected that clinical signs of FMD would be very rapidly detected among the breeding bulls, as the awareness in relation to the disease risk and its symptoms is high among the bull stud's employees and all animals are monitored twice daily to detect any health abnormality. The main sources of uncertainty are the possibility of virus excretion in semen from asymptomatic partially protected bulls as well as virus excretion before the onset of clinical signs.[8]

Where it is present, FMD is a barrier to international trade of live animals and related products including semen. Indeed, if introduced into an FMD-free country, the disease could spread rapidly, disrupting livestock production, causing embargoes by trading partners, and therefore generating important direct and indirect economic losses for the newly infected country.[9] The aim of this study was to estimate the probability for a trading partner to import bull semen contaminated with viable FMD virus from Israel.

2. MATERIAL AND METHODS

2.1. Model Formulation

Based on models that have been previously published,[10–12] a stochastic and dynamic risk assessment model was developed to evaluate the annual likelihood of exporting at least one ejaculate containing viable FMD virus from Israel, hereafter referred to as the risk of release (R). The model was based on the risk pathway presented in Fig. 1, which presents the eight steps leading to the export of a contaminated ejaculate. The probability (P) that a single infected ejaculate is exported is given by:

$$P = P_1 * (P_2 * (P_3 * P_4 + (1 - P_3)) + (1 - P_2)) * P_5 * P_6 * P_7 * P_8, \quad (1)$$

where P_i is the conditional probability associated with step i. The risk of release (R), following a multilevel binomial process, can therefore be calculated using the following expression:

$$R = 1 - (1 - P)^N,$$

where N is the number of ejaculates imported annually by the trading partner. The parameter estimates were obtained from a review of the relevant literature as well as based on data collected from the semen production process at the bull stud. The inputs and conditional probabilities are defined in the following sections and summarized in Table I.

2.2. FMD Outbreak Model

The risk assessment model incorporated the temporal dynamics of the spread of FMD virus within the bull stud. The evolution of an outbreak of FMD within the bull stud was simulated using a stochastic compartmental SLIR (susceptible, latent, infectious, and resistant) transmission model based on the Gillespie algorithm[13] and parameterized with data extracted from the literature.[14,15] The latent stage

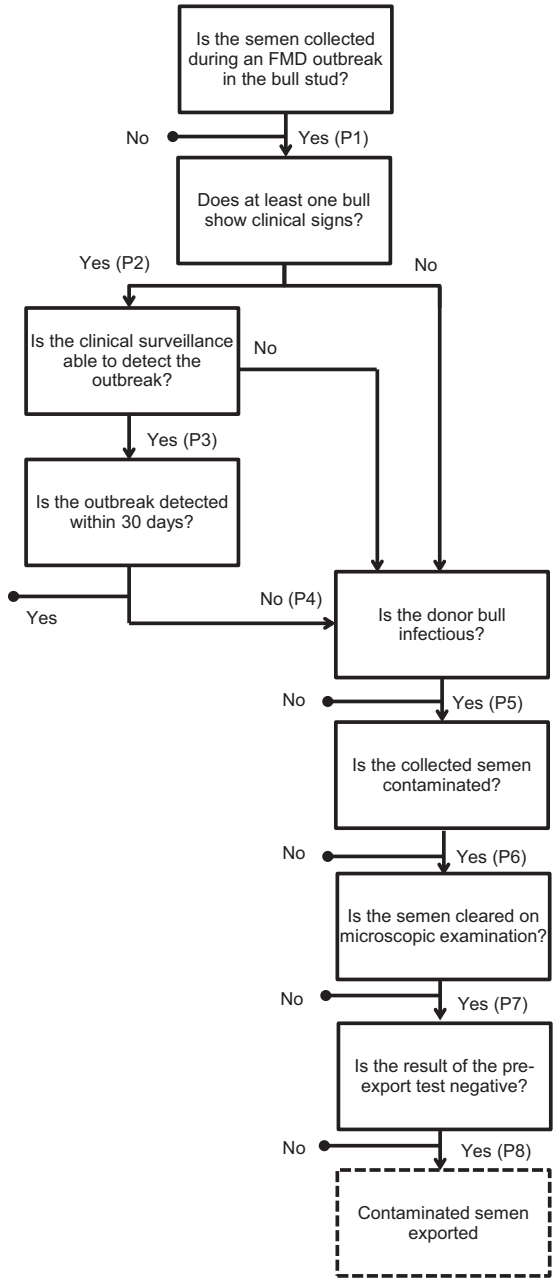

Fig. 1. Risk pathway for the release assessment.

was defined as the period between exposure and the time when the virus becomes detectable in samples taken from the infected animal. This stage was followed by an infectious stage, defined as the period during which the virus is detectable in biological samples and when transmission can occur.[16] This distinction was preferred to the distinction between subclinical and clinical stages as it has been reported that bulls may excrete FMD virus in their semen up to four days before the onset of clinical signs.[17] According to the literature, the duration of the latent and infectious periods was set at four and six days, respectively.[2,16,18] A study of the intraherd transmission of FMD in a 1,000-cow dairy herd reported that the effective daily contact rate could range from 13.7 to 216.0.[15] In our model, the effective daily contact rate was reduced to five. This is because, since the bulls are kept in individual pens, they can have direct contact with only two or three neighbors, although airborne transmission of the virus as well as indirect transmission through fomites[19] may still occur. In a study among Saudi dairy cows vaccinated at least once a year, the within-herd prevalence of FMD clinical cases over the course of an epidemic varied between 0.3% and 53%.[20,21] Another study in vaccinated dairy herds estimated an average within-herd prevalence of FMD infection of 43%.[22] This figure was based on clinical inspection and serology, and therefore included both clinical and subclinical infections. Consequently, as all bulls in the collection center are vaccinated annually, we accounted for the potential lack of vaccine effectiveness by setting the proportion of susceptible bulls in the transmission model at 43%. The transmission model was initiated on day 1 corresponding to the day the virus was introduced into the population of 180 bulls, i.e., one animal has just been exposed and is in the latent stage. The temporal dynamics of the estimated within-herd prevalence $f(t)$, which accounts for subclinically and clinically infected animals (as these groups are both likely to excrete virus in their semen), and the duration of the outbreak t_{max} were extracted for each simulation.

2.3. Evidence Gathering and Parameter Estimation

2.3.1. Is the Semen Collected during an FMD Outbreak in the Bull Stud?

Due to the high biosecurity measures in the bull stud and its location in a low-risk district (only one FMD outbreak in the last 10 years), the risk of introduction of FMD virus into the stud is expected to be lower than in a randomly selected Israeli cattle farm. Because the uncertainty around the estimate of this variable is relatively high, three scenarios were considered for the annual probability of an FMD outbreak in the bull stud (H).

In the worst-case scenario (scenario 1), the probability that the stud experiences an outbreak of FMD

Table I. Inputs Used for the Risk Model

Input	Variable	Distribution or Estimate	Reference
Day of collection	C	Discrete Uniform (0, t_{max})	Not applicable
Duration between the introduction of the virus and the detection of the outbreak (days)	D	Poisson ($\lambda = 17$)	
Annual probability of a foot-and-mouth disease (FMD) outbreak in the bull stud	H	*Scenarios 1 and 2*: Empirical distributions (see data in supplementary material) *Scenario 3*: Pert(0, 0.07%, 0.5%)	Data from IVS
Probability that the semen from infected bull presents abnormal microscopic characteristics	M	Uniform (0, 0.75)	17, 26
Number of ejaculates exported annually	N	*Scenarios A to E*: 500, 200, 100, 50, and 20, respectively	Data from IVS
Proportion of ejaculates undergoing a preexport test	P	*Scenarios α, β, and γ*: 0, 0.5, and 1, respectively	Not applicable
Probability that a semen collection is performed in the collection center during an outbreak of FMD	P_1	$P_1 = \frac{H^* t_{max}}{365}$	Not applicable
Probability that at least one bull in the stud shows clinical signs	P_2	Beta (mean 0.88, *SD* 0.067)	
Probability that an outbreak of FMD in the bull stud is detected	P_3	Pert (0.31, 0.9, 1)	
Probability that the outbreak has not been detected by the time the semen is exported	P_4	$1 - \sum_{i=0}^{30} \frac{\lambda^{i^*} \exp(-\lambda)}{i!}$	Not applicable
Probability that the donor bull is infectious, given that an outbreak of FMD is occurring in the collection center	P_5	$f(C)$	Not applicable
Probability of viral excretion in bull semen	P_6	Beta (mean 0.083, *SD* 0.077)	25, 26
Probability that the semen is cleared for export on microscopic examination	P_7	$1 - M^* S_m$	Not applicable
Probability that the preexport test performed on semen is negative	P_8	$1 - p^* S_p$	Not applicable
Sensitivity of the detection of abnormal semen characteristics	S_m	Uniform (0.5, 1)	Data from IVS
Sensitivity of the preexport test performed on semen	S_p	Uniform (0.9, 1)	31–33

was assumed to match the annual herd incidence in Israel. "H" was modeled using an empirical distribution fitted to the data on incidence of FMD outbreaks in Israeli cattle farms available for the last 11 years (data provided as supplementary material) (IVS, personal communication). This scenario should be considered as the worst-case scenario, as it includes dairy herds as well as beef herds. Because beef herds are usually kept under extensive farming conditions, they are expected to have a much higher risk of FMD outbreaks than dairy herds through contacts with other cloven-hoofed animals, lower vaccination coverage, and less intensive surveillance (IVS, personal communication).

In the intermediate-risk scenario (scenario 2), "H" was modeled using an empirical distribution fitted to data on annual herd incidence of FMD outbreaks in Israeli dairy farms (supplementary material). This is an intermediate scenario, as dairy farms are less at risk than beef farms of contracting FMD (zero grazing, few contacts with other cloven-hoofed animals, high vaccination coverage, and daily surveillance), but they are still more likely to become infected than the semen collection center because of lower biosecurity measures.

In the most plausible scenario (scenario 3), "H" was modeled assuming effective biosecurity measures in the bull stud. This is the most plausible scenario for H, as high biosecurity measures are in place in the facilities. Therefore, introduction of the virus through infected animals (cattle or wildlife), contaminated vehicles, or workers is unlikely. This scenario also takes into account the location of the bull stud in the Kanot Veterinary District, which has experienced only one FMD outbreak in the last 10 years (in 2007). As a consequence of this lower risk, "H" was modeled in this scenario using a Pert distribution whose parameters (minimum, mode, and

maximum) were set as 50% of the minimum, mode, and maximum of the empirical distribution used in scenario 2: 0, 0.07%, and 0.5%, respectively.

P_1, the probability that a semen collection is performed in the collection center during an outbreak of FMD, was given by the following expression:

$$P_1 = \frac{H^* t_{\max}}{365}.$$

2.3.2. Would FMD Virus-Infected Bulls Show Clinical Signs?

It has been reported that vaccinated animals can become infected and excrete virus without presenting clinical signs or presenting only mild clinical signs.[20,22–25] In a study by Cottral as cited by Callis,[25] only two out of seven vaccinated bulls developed clinical signs after experimental infection while none of the 11 vaccinated bulls infected via aerosols from infected pigs developed clinical signs in another study.[26] A study of 25 vaccinated and infected herds in Bolivia reported that 22 herds contained at least one animal with clinical signs.[22] Given these data, the probability that at least one bull in the stud shows clinical signs given that an outbreak occurs (P_2) was modeled as a Beta distribution with mean 0.88 and standard deviation 0.067.

2.3.3. Would the Animal Health Surveillance Detect an Outbreak?

We assumed that once an outbreak has been detected, no semen would be collected until the center has been cleared of FMD virus. In the bull stud, surveillance is based only on the detection of clinical signs (there is no routine laboratory testing of animals). All bulls' health is closely monitored by the staff for typical clinical signs of FMD (pyrexia, ptyalism, lameness, vesicles in the mouth and/or the interdigital spaces).[24] A study of vaccinated dairy herds in Bolivia estimated the sensitivity of detection of FMD based on clinical signs at only 0.31.[22] As the surveillance in the bull stud is more intensive than in dairy herds, the probability P_3 that an outbreak in the collection center is detected, given that at least one bull shows clinical signs, was modeled using a Pert distribution of minimum = 0.31, mode = 0.9, and maximum = 1.

2.3.4. Would the Outbreak be Detected within 30 Days?

We assumed that the bull semen is stored for, at least, 30 days before being exported, as recommended by the OIE Terrestrial Animal Health Code.[27] Hence, we used this value as a worst-case scenario to define the parameter P_4. If the outbreak is detected before the semen is exported, all semen ejaculates collected during the at-risk period would be destroyed. Published data on previous outbreaks in Europe suggest that outbreaks in cattle farms are generally detected between one and five days after the onset of clinical signs.[28,29] Vaccinated animals may express mild clinical signs, which do not immediately lead to FMD suspicion among the staff. This is likely to lengthen the duration between onset of clinical signs and detection. The IVS expected a maximum duration of three days from onset of the clinical signs to detection in a typical vaccinated dairy herd (IVS, personal communication). Although the average duration of the incubation period (infection to first clinical signs) of FMD is often considered to be five days, it may range from one day to up to 14 days.[28] Therefore, the duration (D) between the introduction of the virus and the detection was modeled by a Poisson distribution: with expected value (λ) 14 + 3 = 17 days. The probability (P_4) that the outbreak could not been detected by the time the semen is exported, given that the surveillance system is able to pick up the disease, can be calculated as follows:[30]

$$P_4 = P\ (D > 30 \text{ days}) = 1 - \sum_{i=0}^{30} \frac{\lambda^{i*} \exp(-\lambda)}{i!}.$$

2.3.5. Would the Donor Bull be Infectious?

P_5 is the probability that the donor bull is infectious, given that an undetected outbreak of FMD is occurring in the collection center. Assuming that semen collection can occur at any time between the beginning of an outbreak (day 1) and its end ($t_{\max}$), the duration of the outbreak at the time of semen collection (C) was modeled using a discrete uniform distribution between 1 and $t_{\max}$. P_5 corresponds to the within-herd prevalence of infectious animals at the day of collection C:

$$P_5 = f(C).$$

2.3.6. Would the Collected Semen be Contaminated?

P_6 is the probability that the semen collected contains FMD virus, given that the donor bull is infected. All donor bulls are vaccinated and FMD vaccination is known to reduce the probability of excretion of virus in semen by preventing generalization of the infection from the inoculation site.[8] Although virus was detected in the semen of non-vaccinated bulls following experimental infection in several studies,[17,18] two experimental infection studies of vaccinated bulls (Grunnet, as reported by Callis,[25] and Sellers *et al.*[26]) failed to recover virus from the semen of 10 and 11 subjects, respectively. Therefore, P_6 was modeled using a Beta distribution with mean 0.083 and standard deviation 0.077.

2.3.7. Would the Semen be Cleared on Microscopic Examination?

M is the probability that the semen microscopic characteristics are abnormal, given that the donor bull is infected. Abnormal ejaculates would not be cleared for export. A study showed that three out of four experimentally infected bulls presented abnormal semen characteristics, such as low sperm count, low sperm viability, or high abnormality rate.[17] However, no abnormalities were detected in the sperm of 11 infected vaccinated bulls in a further study by the same authors.[26] Therefore, M was modeled by a uniform distribution of minimum $= 0$ and maximum $= 0.75$.

The quality of semen is systematically monitored at the collection center, thus potential abnormalities induced by FMD infection of the donor bull are very likely to be picked up. No data were available in the literature on the sensitivity of the detection. As the semen ejaculates are evaluated by both an automatic equipment and a lab worker based on viability, morphology, motility, and sperm concentration parameters, we assumed that the sensitivity S_m of the detection of abnormal semen (i.e., the probability to detect an abnormality in an abnormal semen) was medium to high. Therefore, S_m was modeled by a uniform distribution between 0.5 and 1. We also assumed that the specificity (i.e., the probability of not detecting an abnormality in the normal semen) was perfect. Consequently, the probability P_7 that the semen is cleared for export on microscopic examination, given that the donor bull is infected, was given by:

$$P_7 = 1 - M^* S_m.$$

2.3.8. Would the Preexport Test Fail to Detect FMD-Virus-Contaminated Semen?

P_8 is the probability that the preexport test performed on semen is negative, given that the semen is contaminated with FMD virus. As recommended by the OIE Terrestrial Animal Health Code,[27] a preexport test can be performed on the ejaculates to detect the presence of FMD virus. Reverse transcription-polymerase chain reaction (RT-PCR) assays are more sensitive than the other individual tests recommended by the OIE for the detection of FMD virus (antigen enzyme-linked immunosorbent assay and virus isolation).[31] The sensitivity of RT-PCR assays used for the detection of FMD virus in blood and serum is very high.[32,33] In the absence of published evidence on the capacity of these assays to detect FMD virus in semen, the sensitivity of the RT-PCR performed on semen (S_p) at the Kimron Veterinary Institute was modeled using a uniform distribution (minimum $= 0.9$, maximum $= 1$).

This procedure is not currently routinely performed on the ejaculates intended for export. As a consequence, three scenarios, α, β, and γ, were considered, where none, half, and all the ejaculates, respectively, are tested before export. P_8 was given by:

$$P_8 = 1 - p^* S_p,$$

where p is the proportion of ejaculates that are tested and equals 0, 0.5, and 1 in scenarios α, β, and γ, respectively.

The combination of these eight steps would result in the export of an ejaculate contaminated with FMD virus. Published data suggest that dilution and freezing techniques used to preserve ejaculates allow the survival of FMD virus for extended periods of time.[18,34] We assumed that all frozen ejaculates contaminated with FMD virus remain infectious even after one month of storage.

2.3.9. Number of Ejaculates Exported Annually (N)

The number of ejaculates imported by a given client (N) varies between 6 and 200 per year according to data provided by the semen export company. To account for the variability among the different clients and for a potential future increase in demand for Israeli bull semen, five scenarios were considered for the number of ejaculates exported annually: $N = 500$ (scenario A), $N = 200$ (scenario B), $N = 100$ (scenario C), $N = 50$ (scenario D), and $N = 20$ (scenario E).

2.4. Sensitivity Analysis

A sensitivity analysis was conducted to assess individually the effect of the uncertainty of several parameters on the overall risk estimate. The median risk of export R was calculated after increasing or decreasing the mean of the distribution of the parameter of interest (P_2, P_3, P_6, D, M, S_m, and S_p) by one standard deviation.

2.5. Model Environment

Monte Carlo simulation uses repeated random sampling from the input probability distributions to generate a probability distribution of the model output(s).[35] We estimated the distribution of the risk of release by computing 30,000 iterations of the model under each of the 45 scenarios. All calculations were performed using the R software version 3.1.0.[36]

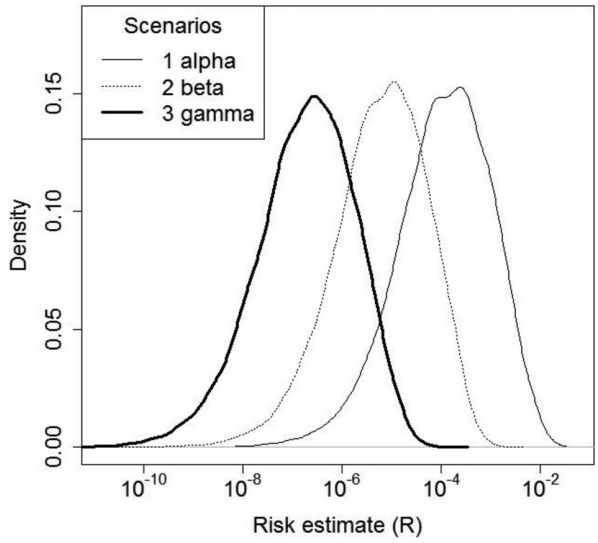

Fig. 2. Distribution of the risk of release (R) under three scenarios, for 100 imported doses.

3. RESULTS

The risk of release R was estimated under 45 different scenarios. Scenarios 1, 2, and 3 correspond to different models for H, from the worst-case scenario to the most plausible. Each of those three scenarios was divided into five scenarios A–E corresponding to the number of ejaculates exported annually (500, 200, 100, 50, and 20, respectively). Each of those six scenarios was further divided into three scenarios α, β, and γ corresponding to the absence of preexport test and to the testing of 50% and 100% of the exported ejaculates, respectively.

For a country importing 100 ejaculates per year and under the most favorable scenario (3γ), where biosecurity measures of the bull stud were taken into account and where all ejaculates underwent a preexport test, the median of the annual probability of exporting FMD-contaminated semen was 0.000013% (Fig. 2 and Table II). Under the least favorable scenario (1α), where the probability of occurrence of an FMD outbreak in the bull stud was assumed to be the annual incidence of FMD outbreak in Israeli cattle farms and where no preexport test was performed, the median value of the risk of release increased to 0.0079% for an import of 100 ejaculates.

The probability that a random exported ejaculate is infected being very small, the risk estimate increases linearly with the number of ejaculates annually purchased by a given client (Fig. 3). In the most plausible scenarios (3γ), the median of the risk increased from 0.0000026% for $N = 20$ ejaculates to 0.000026% and 0.000064% for $N = 200$ ejaculates and $N = 500$ ejaculates, respectively.

The sensitivity analysis showed that the probability that the semen from an infected donor bull contains virus (P_6) had the highest impact on the risk output. The risk increased almost threefold when this parameter was increased by one standard deviation (Fig. 4). The risk decreased by 30–40% when the sensitivity of the preexport test on semen doses (S_p) or the probability that an outbreak of FMD in the bull stud is detected (P_3), respectively, were increased by one standard deviation. Modifying the other parameters had a small impact on the risk output.

4. DISCUSSION

Infection of cows through artificial insemination with semen collected from FMD-vaccinated bulls has never been reported. The United States has been safely importing bull semen from FMD-infected countries for over 20 years. Federal regulations require that all vaccinated donor bulls whose semen shall be imported to the United States present a negative serology test for antibodies against nonstructural proteins (NSP) (vaccination does not induce the production of such antibodies) and a negative virology test on an esophageal-pharyngeal sample.[25] Similarly, in France, semen from vaccinated bulls raised in non-FMD-free areas was used to

Table II. Estimations of the Risk of Release and of the Number of Years before Release of FMD Virus When the Number of Exported Ejaculates is Set at 100 (Scenarios *C*)

Scenarios		Model Estimates			
		Annual probability of export of at least one contaminated ejaculate		Years until one contaminated ejaculate is exported	
Annual probability of a FMD outbreak in the stud (*H*)	Proportion of ejaculates tested by RT-PCR	Median	95th percentile	Median	95th percentile
1 (annual incidence in all cattle farms)	α	$7.9 * 10^{-5}$	$2.6 * 10^{-3}$	12,622	378
	β	$4.2 * 10^{-5}$	$1.4 * 10^{-3}$	24,028	720
	γ	$2.9 * 10^{-6}$	$1.3 * 10^{-4}$	347,904	7,607
2 (annual incidence in dairy farms)	α	$8.0 * 10^{-6}$	$2.6 * 10^{-4}$	125,630	3,837
	β	$4.2 * 10^{-6}$	$1.4 * 10^{-4}$	239,763	7,327
	γ	$2.9 * 10^{-7}$	$1.3 * 10^{-5}$	3,466,212	78,456
3 (50% of annual incidence in dairy farms)	α	$3.5 * 10^{-6}$	$1.0 * 10^{-4}$	282,717	10,026
	β	$1.9 * 10^{-6}$	$5.2 * 10^{-5}$	540,293	19,090
	γ	$1.3 * 10^{-7}$	$5.0 * 10^{-6}$	7,810,330	198,764

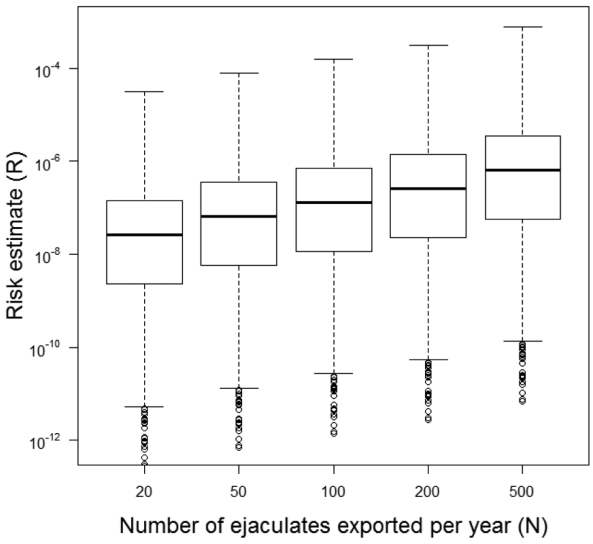

Fig. 3. Influence of the number of ejaculates exported annually on the risk estimate in the most plausible scenario when all ejaculates undergo a preexport test (3γ).

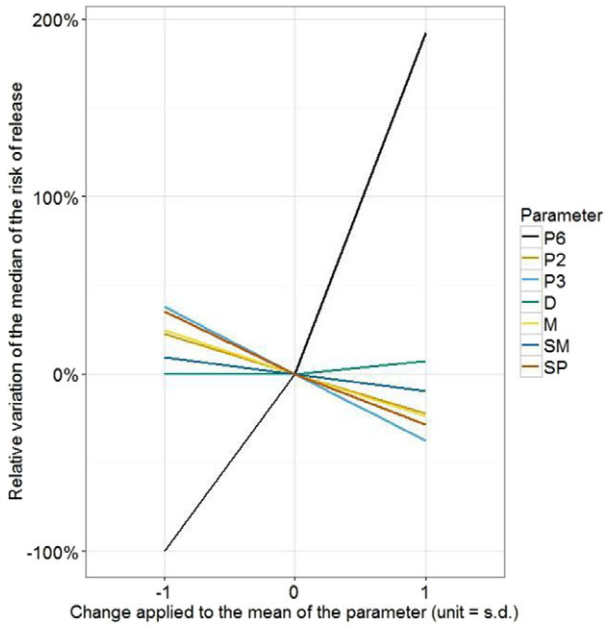

Fig. 4. Results of the sensitivity analysis in scenarios *C* ($N = 100$ doses). For interpretation of the color legend, the reader is referred to the online version of this article.

inseminate nonvaccinated cows in FMD-free areas between 1960 and 1991 without resulting in any FMD outbreak.[37]

There is some debate in the literature about the "carrier" status, i.e., persistently infected animals able to disseminate the virus without displaying clinical signs. There is no formal evidence of carriers transmitting FMD virus to susceptible animals.[38,39] Moreover, it is unlikely that a vaccinated animal becomes a carrier, as the exposure dose needs to be very high for this to happen. As vaccinated animals release very low levels of virus into their environment, it is unlikely that bulls in the stud would become carriers.[40] Although unpublished results from Cottral (cited in Refs. 25 and 40) indicate the case of an unvaccinated bull intermittently excreting FMD virus in semen for up to 42 days, it is highly unlikely that a vaccinated, persistently infected bull would excrete virus in semen due to the protective effect of the circulating antibodies.[38] Therefore, the

probability of having carriers was not considered in this analysis.

In the most plausible scenario of this study, where a client purchased 100 ejaculates per year and they were all tested before export (scenario 3γ), the median of the annual probability of exporting FMD-contaminated semen was estimated at $1.3 * 10^{-7}$ and there was a 95% chance that this annual probability would be below $5.0 * 10^{-6}$. Under this scenario, the median number of years before a contaminated ejaculate is exported is over 7 million and there is a 95% chance that it is over 198,000 years. Under the worst-case scenario (1α) and for the same number of ejaculates, one infected ejaculate would be exported on average every 12,600 years and there is a 95% chance that this value is over 378 years. The results presented here suggest that the risk of release is very low. The decision as to whether this level of risk is negligible and acceptable is up to the importing country. If the risk managers conclude that the risk of release of FMD virus to the FMD-free country following the purchase of bull semen from Israel is not negligible or acceptable, it will be necessary to conduct the exposure and consequence assessments.[41] The final decision should be based on the results of the complete import risk assessment. If such a trade agreement was to be decided, it should involve an audit of the facilities and processes, which would confirm the compliance with the different parameters used in the present assessment, for example, regarding the respect of the 30-day storage duration.

The comparison of scenarios α, β, and γ demonstrates that the implementation of a systematic preexport test on the ejaculates is an effective risk mitigation strategy. For constant levels of parameters H and N, the risk of release is lowered by a factor of 2 when a preexport test is performed on half of the ejaculates and by a factor of 28 when a preexport test is performed on all the ejaculates. The impact of other strategies to reduce the risk of release could be studied by incorporating them into the risk model, as was done with the preexport test. The selection of risk mitigation strategies can also be guided by the sensitivity analysis. Measures that have an impact on the influential input variables are likely to decrease the risk of release significantly. For instance, the clinical surveillance system in place in the bull stud is critical, as a higher probability that an outbreak is detected was shown to decrease the risk estimate significantly. Some variables such as the probability of viral excretion in infected bulls cannot be influenced by any mitigation measure as they depend on the immune response in the infected animals. The disease surveillance system in place could also be further improved by implementing routine NSP serology tests in donor bulls to detect a potential circulation of FMD virus in the bull stud at subclinical levels. The sensitivity analysis showed that the probability of virus excretion in the semen of infected bulls, the probability of detection of an outbreak, and the sensitivity of the preexport test had the largest influence on the risk estimate. The first two parameters were associated to a large uncertainty, due to the limited amount of data available in the literature. Therefore, the risk estimates provided by our study could be improved by incorporating the results of future field and laboratory studies aiming at filling these information gaps.

This study suggests that the risk of release of FMD virus from Israel via export of bull semen is very low, even though Israel is not officially free from FMD. Such results should be considered by decision-makers considering importing bull semen from Israel. Further, the stochastic dynamic risk assessment model developed in this study is sufficiently flexible to be easily adapted to other pathogens and other settings.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary material. Historical incidence of FMD outbreaks in Israeli cattle farms (in-country data collection)