



# Risk factors for infection with Foot-and-Mouth Disease virus in a cattle population vaccinated with a non-purified vaccine in Iran



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## ABSTRACT

In this study, we estimated the level of Foot-and-Mouth (FMD) virus infection in a cattle-dense north-western province of Islamic Republic of Iran and analyzed putative risk factors for FMD infection. Calves (6–24 months of age) from all 17 districts of West Azerbaijan were tested for antibodies against non-structural proteins (NSP-Ab) of FMD virus. A proportional stratification with a minimum of 30 epi-units was applied for 3 different husbandry systems: villages, dairy and mixed farms. Within an epi-unit, 30 calves were sampled. For the interpretation of ELISA test results, we used the 50% inhibition (50PI) cut-off as per producer's instructions and created one at 75% inhibition (75PI) based on the lowest point of the histogram of PI results. This approach resulted in three categories of outcomes; negative (N), low-positive (LP) and high-positive (HP). A generalized mixed-effect model for binary outcomes was used for analysing putative risk factors and was run for both cut-off values.

A total of 8378 calves from 202 villages, 51 dairy farms and 28 mixed farms were eligible for analysis. The percentage of calves testing positive (LP + HP) was 53.7% (95% Confidence interval (CI): 52.6%–54.8%), with 39.6% (95% CI: 38.6–40.7%) testing HP ( $n = 3309$ ) while 14.1% (95% CI: 13.5–15.0%) of calves tested LP ( $n = 1188$ ). Of 281 epi-units sampled, all calves sampled tested negative in only 2 epi-units (0.7% (95% CI: 0.1–2.5%)) and more than 25 calves tested positive in 29 epi-units (10.3% (95% CI: 7.0–14.5%)). Outcomes of regression modelling using the 50 PI cut-off indicated that, for each month increase in age, the odds of testing positive increased 1.01 times (95% CI: 1.00–1.03). The odds of calves testing NSP-positive increased 1.46 times (95% CI: 1.22–1.77) for calves residing in epi-units that had experienced clinical FMD in the 12 months preceding this study. The odds of calves owned by livestock owners who traded livestock testing positive were 1.4–1.6 times higher than those owned by persons not engaged in trading while the odds for calves testing positive in dairy herds was 1.62 (95% CI: 1.10–2.35) times higher compared with calves in villages. The results of the model using the 75 PI cut-off value resulted in comparable estimates, with the age-effect becoming more evident.

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These results have confirmed widespread FMD infection and were used in developing a risk-based control strategy on FMD, in line with Stage 1 of the Progressive Control Pathway for FMD (PCP-FMD).

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## 1. Introduction

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease which affects all cloven-hoofed domestic animals including cattle, sheep, goats, pigs and buffalos (OIE, 2008). Recently the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) jointly launched a global FMD control strategy which consists of three components (FAO, 2012a). The first component is directly related to FMD control and advocates the use of the Progressive Control Pathway (PCP-FMD), developed by the European Commission for the Control of FMD (EuFMD) in collaboration with FAO and OIE (Sumption et al., 2012). The PCP-FMD is a framework that consists of 6 Stages and 5 steps, going from an endemic, unknown FMD situation (Stage 0) to an official OIE status of FMD-free with (Stage 4) or without (Stage 5) vaccination. In PCP-FMD Stage 1, activities are undertaken to enhance understanding of the epidemiology of FMD and to develop a risk-based approach to reduce the impact of clinical FMD. Among these activities is describing the distribution of FMD virus in the country, a critical component. It emphasizes the usefulness of serological studies in addition to clinical outbreak investigation, as the former includes subclinical or unreported cases of FMD in the surveillance system. The final outcome of PCP-FMD Stage 1 is the development of a risk-based strategic plan (RBSP), taking into account that resources such as time, manpower and finances are limited. To develop an RBSP, countries must identify the highest risks for FMD entry and/or spread within the country. Through risk assessment techniques such as the development of risk pathways, measures to mitigate the risk can be identified, resulting in a strategy targeted to the particular risks of the country (FAO, 2012b).

FMD is endemic in Islamic Republic of Iran (for readability further referred to as Iran) and in countries bordering it (Turkey, Iraq, Afghanistan and Pakistan (WAHID, 2013). Three of the 7 FMD serotypes have been circulating in Iran since 2011: serotypes O, A, and Asia1 (World Reference Laboratory for FMD, 2013; FAO, 2013a; FAO, 2013b; Gilbert et al., 2005; Jamal et al., 2010). Until 2011, control of FMD in Iran was mainly focused on mass vaccination of susceptible livestock. A non-purified tetravalent vaccine produced locally by the Razi Institute (Tehran, Iran) was used, containing serotypes A, Asia1 and two strains of serotype O. The vaccine producer informed the Iranian Veterinary Organization (IVO) that the vaccine provided immunity for 4 months. Based upon this information, IVO had a policy to vaccinate against FMD three times a year for cattle and once a year for small ruminants. Application of booster vaccination (re-vaccinating primo-vaccinates after 3–6 weeks) was not part of the policy.

In a recent FAO-EuFMD project (Combating foot-and-mouth disease through enhanced and co-ordinated

surveillance activities; Phase 3 of the FMD surveillance centre initiative), Iran was supported to enhance its FMD surveillance activities in line with the PCP-FMD framework. In this project, special focus was placed on the province of West Azerbaijan. West Azerbaijan is a livestock-dense province in the northwest of Iran bordering with Iraq, Turkey and Azerbaijan. It is considered an important gateway for informal livestock trading with Turkey. In 2011, there were an estimated 3.3 million cattle and 500,000 small ruminants in West Azerbaijan, with 90% of livestock housed in approximately 3500 epidemiological-units (villages, commercial dairy herds and commercial mixed herds (combination of sheep, goats, beef and or dairy)). The remainder of livestock was managed in nomadic herds. Approximately 4 million doses of vaccine were administered in West Azerbaijan in each of 2009 and 2010. However, in 2011, only around 2.2 million doses were applied due to limited production capacity of the Razi Institute. This meant that effectively few epi-units received 3 vaccinations in the year prior to this study.

There were 112 outbreaks of clinical FMD notified in 2009, 321 outbreaks in 2010 and 313 outbreaks in 2011 in West Azerbaijan province alone (GISVET database, IVO), resulting in an annual FMD incidence rate between 3.4% and 9.2% of epidemiological-units. Even with these numbers, it can be assumed that there was considerable bias in FMD outbreak reporting and that FMD virus transmission may have gone unnoticed in this endemic situation and with variable vaccination coverage (McLaws et al., 2012; Sumption et al., 2008).

To accurately describe the FMD virus distribution, insight into the actual levels of FMD infection in the livestock population was needed to complement the outbreak reports of clinical disease. In our paper, we present the results from a serological cross-sectional study in West Azerbaijan with the objective to estimate prevalence of antibodies to non-structural proteins in young cattle, indicating recent FMD infection, and to assess putative risk factors for FMD infection.

## 2. Method and materials

### 2.1. Study population

As the focus of this study was to learn about FMD infections that were reflecting recent introductions, the target population was calves between 6 and 24 months of age, from the 17 districts of West Azerbaijan province, Iran.

### 2.2. Study design

Multi-stage sampling was applied with the primary unit of interest being a village or herd (referring to commercial

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