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Randomised field trial to evaluate serological response after foot-and-mouth disease vaccination in Turkey

T.J.D. Knight-Jones[a,b,*], A.N. Bulut[c], S. Gubbins[a], K.D.C. Stärk[b], D.U. Pfeiffer[b], K.J. Sumption[d], D.J. Paton[a]

[a] The Pirbright Institute, Pirbright, UK
[b] The Royal Veterinary College (VEEPH), University of London, UK
[c] The Şap institute, Ankara, Turkey
[d] The European Commission for the Control of FMD, FAO, Rome, Italy

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ABSTRACT

Despite years of biannual mass vaccination of cattle, foot-and-mouth disease (FMD) remains uncontrolled in Anatolian Turkey. To evaluate protection after mass vaccination we measured post-vaccination antibodies in a cohort of cattle (serotypes O, A and Asia-1). To obtain results reflecting typical field protection, participants were randomly sampled from across Central and Western Turkey after routine vaccination. Giving two-doses one month apart is recommended when cattle are first vaccinated against FMD. However, due to cost and logistics, this is not routinely performed in Turkey, and elsewhere. Nested within the cohort, we conducted a randomised trial comparing post-vaccination antibodies after a single-dose versus a two-dose primary vaccination course.

Four to five months after vaccination, only a third of single-vaccinated cattle had antibody levels above a threshold associated with protection. A third never reached this threshold, even at peak response one month after vaccination. It was not until animals had received three vaccine doses in their lifetime, vaccinating every six months, that most (64% to 86% depending on serotype) maintained antibody levels above this threshold. By this time cattle would be >20 months old with almost half the population below this age. Consequently, many vaccinated animals will be unprotected for much of the year. Compared to a single-dose, a primary vaccination course of two-doses greatly improved the level and duration of immunity. We concluded that the FMD vaccination programme in Anatolian Turkey did not produce the high levels of immunity required. Higher potency vaccines are now used throughout Turkey, with a two-dose primary course in certain areas.

Monitoring post-vaccination serology is an important component of evaluation for FMD vaccination programmes. However, consideration must be given to which antigens are present in the test, the vaccine and the field virus. Differences between these antigens affect the relationship between antibody titre and protection.

1. Introduction

As the duration of FMD vaccine protection is short lived, animals require regular revaccination. In Turkey cattle are routinely vaccinated twice a year [1–4]. It is recommended that after initial vaccination at two months of age, cattle receive a second dose one month later. However, as mass vaccination is costly [5], some countries, including Turkey, use a single-dose primary vaccination course.

Much is known about immunity after a single dose of high potency vaccine used to control outbreaks in free countries [6–10]. However, requirements in this setting differ to the sustained protection required in endemic countries where standard potency ($\geq$3PD$_{50}$) FMD vaccines are typically used (PD$_{50}$ $\equiv$ 50% protective dose). Limited protection after a single dose of $\geq$3PD$_{50}$ FMD vaccine is not uncommon [4].

FMD structural protein (SP) antibody levels are strongly correlated with protection [11–18]. In this prospective field study, we assessed post-vaccination SP antibody levels in a cohort of cattle, vaccinated within the Turkish FMD vaccination programme, the

* Corresponding author at: Present address: International Livestock Research Institute (ILRI), c/o WorldFish, Lusaka, Zambia. Tel.: +260 211 294065.
E-mail addresses: t.knight-jones@cgiar.org, tknightjones@gmail.com (T.J.D. Knight-Jones).

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objective being to evaluate vaccine protection in the population at large. A randomised trial, with two parallel arms was nested within the cohort to assess the effect of administering two vaccine doses approximately one to two months apart as opposed to a single dose.

2. Materials and methods

2.1. Study design and sampling

2.1.1. Background and village selection

Households were selected from an FMD sero-prevalence survey conducted in Anatolian Turkey in September–November 2012 ("autumn"). We present results of the prospective study only and not the sero-prevalence survey. In the survey, cattle were randomly sampled from each of 1027 villages, randomly selected across Turkey, stratified by region, using the national livestock database as a sampling frame.

Villages in Central and Western Anatolia conducted routine FMD vaccination immediately after sampling. Prospective study eligibility was restricted to villages that vaccinated one to two months before December 2012 ("winter") for which serology results were available. From these 37 villages, four were inaccessible due to heavy snow, one could not be sampled as cattle were at grazing and a further nine villages were excluded due to inadequate vaccination records. This left 98 households in 23 villages, from eight provinces, included in this prospective study (see Fig. 1).

2.1.2. Sampling

Each household was visited in December 2012 ("winter") and again in late February or early March 2013 ("spring"). During December, all cattle <24 months old present at enrolled households were sampled, including those not sampled in the autumn sero-prevalence survey. Those that tested positive for non-structural protein (NSP) antibodies at autumn sampling, indicating prior infection, were excluded. Vaccines used were purified for NSP proteins, so, unlike infection, vaccination rarely leads to NSP sero-positivity. This differs from SP antibodies which are produced after infection or vaccination. Of 736 animals sampled during winter, 355 had been sampled in autumn 2012. Animals were identified by unique ear-tag numbers, something all Turkish cattle should have.

2.1.3. Booster allocation

At winter sampling, half the cattle in each household were given an additional dose of Şap institute trivalent FMD vaccine. Animals within a household were divided into two equally sized groups, balanced in age and prior FMD vaccination status. One group was then randomly selected to receive an additional dose of vaccine if the last ear-tag digit of the first animal selected was <5; the other group received no additional vaccination. Animals under two months of age were not vaccinated. Animals not previously vaccinated were randomised separately with one-in-four selected for vaccination.

2.1.4. Additional information

Farmers and investigators were present during vaccination and were not blinded. Outcomes were serological and those conducting the laboratory tests were blinded from the details of the animals being tested. Study data were only available to T.J.D.Knight-Jones. Animal housing and location remained unchanged throughout the study.

2.1.5. Vaccination and sampling procedures

During autumn, animals were sampled and vaccinated by state veterinary staff. Winter and spring sampling was conducted by T.J.D.Knight-Jones, A.N.Bulut and M.Alkan, when animals were briefly examined and blood sampled, with additional vaccination for selected animals. Animal and holding details were collected, including information on prior vaccination, disease, trading and husbandry. All animals were permanently housed during the study with turnout for grazing commencing shortly after final sampling.

2.1.6. Vaccines

The $\geq$3 PD_{50}, NSP purified Şap institute (Ankara, Turkey) trivalent FMD vaccine, contains strains O Panasia II (O Tur 07), A Iran-05 (A TUR 06) and Asia-1 Sindh-08 (Asia-1 TUR 11). Six different vaccine batches were used in autumn vaccination. A single batch was used within a province, with 2 ml injected intra-muscularly for each dose. For all winter vaccination a single batch was used.

2.1.7. Serology

Sera were tested for NSP antibodies (PrioCHECK FMDV NS ELISA-Prionics, Zurich, Switzerland). Sera were also assessed for SP antibodies for the vaccine serotypes using the liquid phase blocking ELISA (LPBE), supplied by The Pirbright Institute, UK. The strains of virus used to produce the ELISA antigens (O Manisa, A22 IRQ 24/64 and Asia-1 Shamir) could not be changed and were different to the strains used to produce the vaccine. These differences were antigenically significant, based on serological matching tests (WRLFMD, The Pirbright Institute).

Sera taken during autumn 2012 sampling were tested for SP antibodies using a single dilution of $1{:}10^2$. This titre is associated with approximately 70% clinical protection [19], assessed by the vaccine manufacturer and other published studies, the latter using a homologous test system [20,21]. Sera collected at winter and

Fig. 1. Map of Turkey showing the location of villages included in the study. As mass vaccination was not conducted in Eastern Turkey in autumn 2012 sampled villages come only from Central and Western Turkey.