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Efficacy of Newcastle disease vaccines and vaccination protocols commonly used in Mali, West Africa

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Newcastle disease, caused by Avian paramyxovirus -1 (APMV-1), is a devastating disease of poultry that is endemic in many countries worldwide. Several commercial vaccines and protocols are available against the disease and in most cases, they provide good clinical protection. However, despite vaccination, cases of Newcastle disease are often seen in some countries, including Mali in West Africa. Although there is only one serotype of APMV-1, eighteen different genotypes have been identified to date. The vaccine strains used in Mali belong to either genotype I (for example I-2) or II (for example LaSota and Hitchner B1) while the most recently identified isolates in Mali are from genotypes XVII and XVIII. This study was therefore undertaken to determine whether, four currently used vaccination protocols in Mali were able to protect chickens against challenges with a recently isolated genotype XVIII strain from Mali (ML008/09) and genotype IV Herts/33. The results showed clinical protection of the vaccinated birds with no shedding of ML008/09 for all of the vaccination protocols used, while shedding was detected in birds challenged with Herts/33. The data generated in this study will assist those working in the area of Newcastle disease management and control in Mali.

Key words: Avian paramyxovirus-1, Newcastle disease, serology, vaccine, Mali.

INTRODUCTION

Newcastle disease (ND) is one of the most important poultry diseases globally due to the substantial losses it cause in the poultry industry. It is a reportable disease to the World Organization for Animal Health (OIE) and is endemic in many countries in Asia and Africa. The causative agent of this disease is the Avian Paramyxovirus-1 (APMV-1), also known as Newcastle disease virus (NDV).

Vaccination is currently the most effective means to control the disease and programmes are regularly implemented in countries in Africa and Asia (Dimitrov et al., 2017). Despite the existence of several genetic variants, all 18 APMV-1 genotypes belong to a single serotype (Diel et al., 2012). Vaccines are based on

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lentogenic viruses mainly from genotypes I and II and are currently used in the field as live or inactivate vaccines. Given that the ND vaccines are produced with strains are more than 70 years old; and due to the rapid evolution AMPV-1, there is often a notable genetic divergence between viruses responsible for outbreaks and the vaccines available to control the disease (Dortmans et al., 2012; Dimitrov et al., 2016). Despite this, some authors have demonstrated that these vaccines can still protect chickens from disease even though they do not prevent viral excretion when chickens are infected by heterologous isolates (Samuel et al., 2013; Roohani et al., 2015; Wajid et al., 2018).

Nevertheless, well-vaccinated commercial farms in many endemic countries in Africa and Asia have reported outbreaks of ND (Ezema et al., 2009). In addition, ND vaccines are believed by many to be responsible for a progressive reduction in the control of infection, virus replication and shedding of viruses (Miller et al., 2007, Samuel et al., 2013). The aim of this study is, therefore, to evaluate the efficacy of current ND vaccination protocols and vaccines used in Mali against currently circulating XVIII genotype viruses.

MATERIALS AND METHODS

Experimental premises

Animal experiments were carried out in the Central Veterinary Laboratory (CVL) facilities in Bamako, Mali. The facilities complied with the biosafety standards recommended by the Terrestrial manual, chapter 2.3.14 (OIE, 2012). Five rooms were chosen for the experiments in order to have one room per animal group. Each room was strictly isolated from the others in order to avoid inter-hall and environmental contamination.

Animals and groups

Female, 45 day old, locally produced chickens (*Gallus gallus*) were used in the study. All of the chickens were confirmed to be seronegative to APMV-1 by hemagglutination inhibition HI assay using standard methods (OIE, 2012) prior to experimentation. In total, 111 chickens were used (55 in the first trial and 56 in the second one). The chickens were randomly divided into five groupsand assigned to the vaccination protocols as described in Table 1.

Vaccines

The characteristics of the different vaccines used in the trials are presented in Table 2. The AVIVAC I-2 vaccine is a thermostable vaccine (Illango et al., 2005; Bensink and Spradbrow, 1999) produced by the Central Veterinary Laboratory in Mali. The vaccine batch N°001 was controlled for quality by the Pan African Veterinary Vaccine center (PANVAC) and tittered at $10^{6.3}$/dose (Young et al., 2012). The vaccine production procedure has been previously described by ACIAR (OIE, 2012; Gallili and Ben-Nathan, 1998). The other vaccines (for example Ita-New, Inmugale® Hitchner B1 and Hipraviar ®) are commercially available in Mali and commonly used in the field against Newcastle disease. In each group vaccination was administered at three time points seven days apart.

Challenge strains

All chickens from Groups 3, 4 and 5 were challenged one week after the 3rd vaccination on day 74. The challenge strains used were ML008/09 (genotype XVIII) isolated in Mali in 2009 (de Almeida et al., 2013) for Trial I and Herts/33 (genotype IV) for Trial II (Table S1). Both viruses tittered at 10^6 of 50% Egg Infective Dose (EID_{50})/ml. The intracerebral pathogenicity index (IPIC) of ML008/09 and Herts/33 were 1.8 and 1.9, respectively (de Leeuw et al., 2005). The viruses were administered intramuscularly in a volume of 100 µl/chicken. The Intramuscular pathway is used to make the challenge as envisaged by the EU pharmacopoeia. This route was used to be sure that the subjects received the full dose. The challenge is done one week after the third vaccination (Table 3).

Clinical monitoring and sampling

After the challenge, the animals were observed daily for fourteen days and all cases of morbidity and mortality were recorded. All suffering chicken were humanely sacrificed. Necropsies were carried out on dead or sacrificed birds to evaluate lesions due to Newcastle disease and to take samples of the affected organs. Cloacal and tracheal swabs were collected from each chicken, before and after vaccinations and challenge, on day 0, 3, 5, 7, 10 and 14 to measure viral shedding.

Analysis of viral excretion by RT qPCR

The real-time polymerase chain reaction (RT PCR) and quantitative real-time polymerase chain reaction (RT qPCR) assay described by de Almeida et al. (2013) was used for the detection of Newcastle disease virus in swabs and organs. In order to transform the Cut-off threshold (Ct) values obtained by RT qPCR into equivalent EID_{50}/ml, a standard dilution range (10^{-3}; 10^{-4}; 10^{-5} and 10^{-6}) of the Queensland V4 vaccine strain with a titter of $10^{8.3}$ EID_{50}/ml was used as a reference. The extraction of the RNA was carried out from a 1 in 10 dilution of the viral stock. The results of excretion were confirmed by sequencing the RT-PCR amplified fragments based on the difference between the cleavage site of strain M008/09 (^{112}RRQKR↓F^{117}) and the reference Herts/33 strain (^{112}RRQRR↓F^{117}) (Alexander et al., 2006).

Viral isolation and evaluation of virulence of excreted strains

Viral isolation on 9 to 11-day-old embryonic SPF eggs (Cerveloup, France) was performed on the positive RT qPCR swabs samples, according to the protocol recommended by the OIE (OIE, 2012). Two hundred microliters of the dilution of each supernatant were inoculated into the allantoic cavity of five eggs. Then, the eggs were incubated at 37°C for 7 days and observed daily. On the following days, the allantoic fluids of the eggs containing dead embryos were recovered and analysed. The embryos that remained alive were sacrificed day 7 and the allantoic fluid was taken for a second cycle of amplification on a new series of eggs. The mortality recorded in the inoculated eggs made it possible to assess the presence of virulent virus. The presence of APMV-1 in the allantoic fluids from the dead embryos was confirmed by RT qPCR as described above.

Serology

Blood samples were taken on day 0 before challenge and on day 3, 5, 7, 10 and 14 post challenges from wing veins of the chickens. The antibody titer of the sera was measured using the ID Screen® Newcastle Disease Indirect Conventional Vaccines kit according to

Table 1. Experimental protocol by group.

Group	Vaccination protocol	Challenge	Number of chickens
1	None	No	15* and 16**
2	None	Yes	10
3	«Avivac I-2» in three applications***	Yes	10
4	« Ita New and Inmugale® V.P. HB1 » Combined in three applications***	Yes	10
5	« Inmugale® V.P. HB1 » in two applicationsand «Hipraviar®-S » in one application	Yes	10

* First trial; ** Second trial; *** One week of interval between vaccinations.

Table 2. Characteristics of the vaccines used in the trials.

Commercial name	Vaccine type	Strain	Route of administration	Individual dose (EID_{50})	Genotype	Manufacturer
Ita-New	Inactivated oil	LaSota	Subcutaneous	10^8	II	Laprovet, France
Inmugale VP® Hitchner B1	Freeze-dried	Hitchner B1	Subcutaneous	10^6	II	CEVA, USA
Hipraviar® -S	Freeze-dried	LaSota	Ocular-nasal	$10^{6.3}$	II	Injevel,Spain
Avivac I-2	Freeze-dried	I-2	Ocular-nasal	10^6	I	CVL/Mali

Table 3. Chronology of the vaccine trial according age of the subjects.

Days	Groups				
	1	2	3	4	5
0-45	Chicken acclimatisation				
45	-	-	Avivac I-2	Ita New + Inmugale V.P. HB1	Inmugale V.P. HB1
52	-	-	Avivac I-2	Ita New + Inmugale V.P. HB1	Inmugale V.P. HB1
59	-	-	Avivac I-2	Ita New + Inmugale V.P. HB1	Hipraviar-S
74	-			Challenge*	
74-87	Clinical monitoring				

* ML008/09 was used for Trial I and Herts/33 strain for Trial II**; -** No vaccine administrationand No challenge.

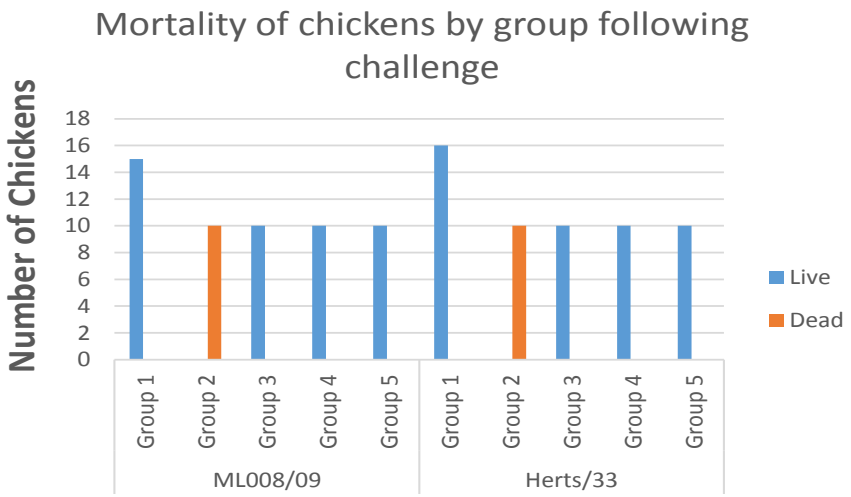

Figure 1. The effect of chicken vaccinated with the LaSota, Hitchner B1 and I-2 strains against the challenge viruses.

the manufacturing recommendations. The coefficient of variation (CV) was calculated from the antibody titers for the vaccinated groups in order to assess the homogeneity of the immune responses induced. The calculation was done according to the following formula:

$$\text{Coefficients of variation} = \frac{\text{Standard deviation}}{\text{Mean of titers}} \times 100$$

The variation threshold is considered as being heterogeneous (undesirable) when the CV is greater than or equal to 50%. A CV < 30%, indicates a more uniform (desirable) response to vaccination (Cherif et al., 2010).

Statistical analysis

Statistical analysis was performed using Stata 14.1 software. The regression analysis was used to check the significant difference ($p \leq 0.05$) between the different groups (protocols) and vaccines against the mortality and viral shedding after due challenge strains.

RESULTS

Clinical protection of vaccinated chickens

The protection of chickens vaccinated with the LaSota, Hitchner B1 and I-2 strains against the challenge viruses (that is ML008/09 and Herts/33) at titers of 10^6 EID_{50}/ml was determined by the absence of morbidity and mortality. After challenge, all chickens in the three vaccinated groups remained clinically normal and no mortality was observed up to the end of the experiment (14 days post challenge) (Figure 1). In contrast, all chickens from Group 2 (that is unvaccinated and challenged control group), showed clinical signs of Newcastle disease for both challenge viruses. Logistic analysis indicates that the probability (odds) of dying is 12 times higher in unvaccinated groups than in vaccinated groups. Viral excretion is higher in unvaccinated group 2 than in vaccinated groups. No unvaccinated and unchallenged animals (Group 1) showed clinical signs of Newcastle disease. Statistical analysis showed a significant difference between groups and vaccines according to mortality ($p=0.000 < 0.05$) for the both challenge viruses (Tables S2, S3 and S4).

Virus excretion

Virus excretion of Herts/33 was detected by RT qPCR in the challenged vaccinated groups. Seven birds had transient and weak tracheal excretion at day 3 post-challenge: (2/10 Group 3), (1/10 Group 4) and (4/10 Group 5) (Table 4). In contrast, no viral excretion was detected in the vaccinated groups challenged with ML008/09 (Table 4). Virus excretion was detected in nine unvaccinated animals challenged with ML008/09 and two unvaccinated animals challenged with Herts/33 strain (Table 4).

Isolation of excreted challenge strains

Both challenge viruses were isolated from all RT qPCR positive chickens from Group 2 (unvaccinated and challenged). In addition, Herts/33 was isolated from two vaccinated and challenged birds, one from Group 3 (H367T) and one from Group 5 (H375T). Analysis of the F gene cleavage site confirmed the identity of both viruses (Figure S1).

Serology

The antibody titers (log_{10}) in Groups 1, 2, 3, 4 and 5 are shown in Figure 2. The threshold for antibody titer positivity

Table 4. Evaluation of virus excretion by RT qPCR.

Challenge strain	Group	Chickens	Swab type	Ct			EID_{50}/ml		
				Day 3	Day 5	Day 7	Day 3	Day 5	Day 7
		AC312C	Cloacal	26.15	ND	ND	2.52×10^4	-	-
		AC312T	Tracheal	22.06	ND	ND	2.535×10^5	-	-
		AC313C	Cloacal	26.15	24.92	23.53	2.52×10^4	5.05×10^4	1.1×10^5
		AC313T	Tracheal	25.13	24.26	26.60	4.475×10^4	7.35×10^4	1.955×10^5
		AC314C	Cloacal	24.43	ND	ND	6.65×10^4	-	-
		AC314T	Tracheal	25.47	ND	ND	3.685×10^4	-	-
		AC315C	Cloacal	27.07	23.70	ND	1.495×10^4	1×10^5	-
		AC315T	Tracheal	25.65	23.92	ND	3.345×10^4	8.85×10^4	-
		AC317C	Cloacal	25.42	ND	ND	3.795×10^4	-	-
ML008/09	2	AC317T	Tracheal	23.75	ND	ND	9.75×10^4	-	-
		H360C	Cloacal	26.17	25.05	ND	2.495×10^4	4.68×10^5	-
		H360T	Tracheal	25.19	22.26	ND	4.33×10^4	2.265×10^5	-
		H361C	Cloacal	25.56	ND	ND	3.515×10^4	-	-
		H361T	Tracheal	23.75	ND	ND	9.75×10^4	-	-
		H362C	Cloacal	24.64	ND	ND	5.9×10^4	-	-
		H362T	Tracheal	24.02	ND	ND	8.4×10^4	-	-
		H363C	Cloacal	24.19	22.08	ND	7.6×10^4	2.495×10^5	-
		H363T	Tracheal	22.09	21.37	ND	2.49×10^5	3.735×10^5	-
		H364C	Cloacal	25.49	23.18	ND	3.645×10^4	1.35×10^5	-
		H364T	Tracheal	24.11	22.76	ND	7.95×10^4	1.705×10^5	-
		H396C	Cloacal	23.63	ND	ND	1.045×10^5	-	-
	2	H396T	Tracheal	23.07	ND	ND	1.43×10^5	-	-
		H397C	Cloacal	20.98	ND	ND	4.645×10^5	-	-
		H397T	Tracheal	23.08	ND	ND	1.42×10^5	-	-
Herts/33	3	H366T	Tracheal	33.39	32.76	ND	1.81×10^2	2.63×10^3	0
		H368T	Tracheal	29.83	29.89	ND	1.52×10^4	1.465×10^4	0
	4	AC361T	Tracheal	34.10	ND	ND	2.77×10^2	0	0
		A940/D436T	Tracheal	32.48	ND	ND	3.12×10^2	0	0
	5	H515T	Tracheal	30.49	ND	ND	1.03×10^2	0	0
		H517T	Tracheal	31.65	ND	ND	5.10×10^2	0	0

ND: Not determined.

was ≥ 993 (Brown et al, 1990). With one exception in Group 4 (H396), the antibody titers were above the threshold in the vaccinated groups (3, 4 and 5) and below the threshold in the unvaccinated Groups (1 and 2) (Cvelic-Cabrilo et al., 1992). More specifically, the means titers (± standard deviation) for undiluted sera were 78.84 ± 58.98 for Group 1, 70.31 ± 43.41 for Group 2, 10,652.72 ± 2 318.63 for Group 3, 13,195.87 ± 527.11 for Group 4 and 7,953.41 ± 1, 753.81 for Group 5. When the sera were diluted, the sera from Groups 3 and 5 were negative for anti-NDV antibodies at a 1/64 dilution while those from Group 4 (vaccinated with Ita-New and Inmugale) were negative between dilutions 1/1024 and 1/4096, indicating a better protection in this group. The CV for Group 1, 2, 3, 4 and 5 were 74.81, 61.74, 21.77, 3.99 and 22.05%, respectively.

The CV values for Groups 3, 4 and 5 were <30%; indicating a homogeneous (desirable) post-vaccination immune response against Newcastle disease at the time of the challenge (Figure 2).

Statistical analysis

Statistical analysis revealed a significant difference in the protocols and vaccines used against mortality from challenge viruses. Vaccines and protocols have protected against mortality from challenge viruses (Tables S2, S3 and S4). A significant effect of mortality was found in Group 2. On the other hand, no significant effect of mortality was found in the vaccinated Groups (3, 4 and 5). In vaccinated groups, the vaccines and protocols protected against the challenge viruses (Table S3).

DISCUSSION

The aim of this study was to evaluate the protection of chickens induced by Newcastle disease vaccine strains

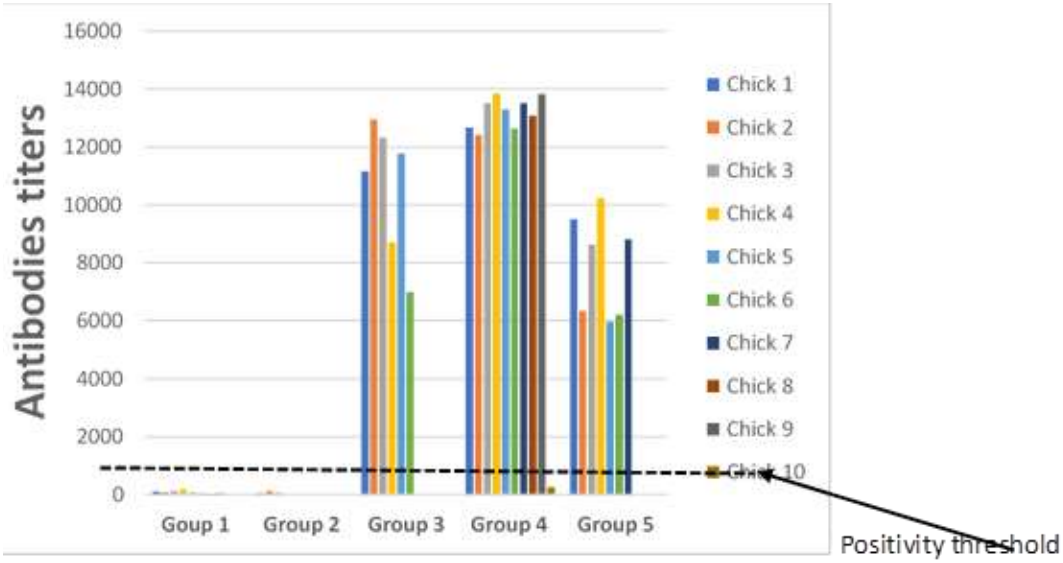

Figure 2. Newcastle disease virus antibodies titers per group before challenge.

(La Sota, Hitchner B1 and I-2) and the vaccination protocols commonly used in Mali against two virulent strains, ML008/09 and Herts/33. During the 14 days of observation after the challenges, none of the vaccinated groups showed any symptoms of Newcastle disease indicating that the vaccine strains, LaSota, Hitchner B1 and I-2, used under the experimental conditions of this study conferred a clinical protection of 100%. Moreover, the three protocols used were able to prevent viral shedding by the birds challenged with the heterologous genotype XVIII ML008/09 strain.

Viral excretion (mostly tracheal) was observed in this study after challenge with the Herts/33 strain in all vaccinated groups despite the induced clinical protection. Birds vaccinated first with a live HB1 strain (2 doses) followed by a live LaSota strain (1 dose) (Group 5) seem less protected against viral shedding; 40% excreted virus compared to 10% in Group 4 which received a live HB1 and an inactivated LaSota strain (3 doses) simultaneously. This difference could be explained by the higher antibody titers observed in the Group 4 birds. More effective protection was observed in Group 4 were also confirmed by the CV value (3.99%) for this group which was less than the CV calculated for Groups 3 and 5 (21.77 and 22.05%, respectively). Better performance of the vaccination protocol applied in Group 4 may be associated with a more effective formulation of the Ita-New vaccine with its combination of two viral strains (LaSota and Hitchner B1) applied in three doses compared to the administration of the viruses individually in the vaccine protocols used for Groups 3 and 5.The phylogenetic distance between genotype XVIII, to which the ML008/09 challenge strain belongs, and the vaccine genotypes I and II is greater than that between the later and the genotype IV pertaining the Herts/33 (Figure 3) (Maminiaina et al., 2010). It is surprising, then, that virus excretion has been observed with Herts/33 strain but not with ML008/09 strain. This observation is unexplained.

Our results suggest that the vaccines and vaccination protocols commonly used against Newcastle disease in Mali are capable of conferring protection against the morbidity and mortality of Newcastle disease under experimental conditions. Whether this is the case under field conditions, needs to be determined. Under field conditions, protection failures can be intensified if chickens are vaccinated at the same time against several viruses or if the vaccine dose is low and/or poorly administered (Nakamura et al., 2014; Miller et al., 2013; Dortmans et al., 2014; Susta et al., 2015). Poor immunization practices and/or immune surpressing opportunistic infections (For example Infectious Bursal Disease, Infectious Bronchitis and parasitosis) may also be responsible for poor immunization of animals with associated viral shedding and spread (Dortmans et al., 2014). Since the vaccination protocol using simultaneously a live HB1 and an inactivated LaSota strain in 3 doses has shown the best performance in virus shedding controlling. This protocol is recommended among those currently used in Mali against Newcastle disease. In any case, the design of new vaccines or vaccination protocols able to completely block virus excretion of all NDV genotype strain is still needed for an optimised Newcastle disease control in affected countries.

ETHICAL APPROVAL

All animal experiments were conducted according to

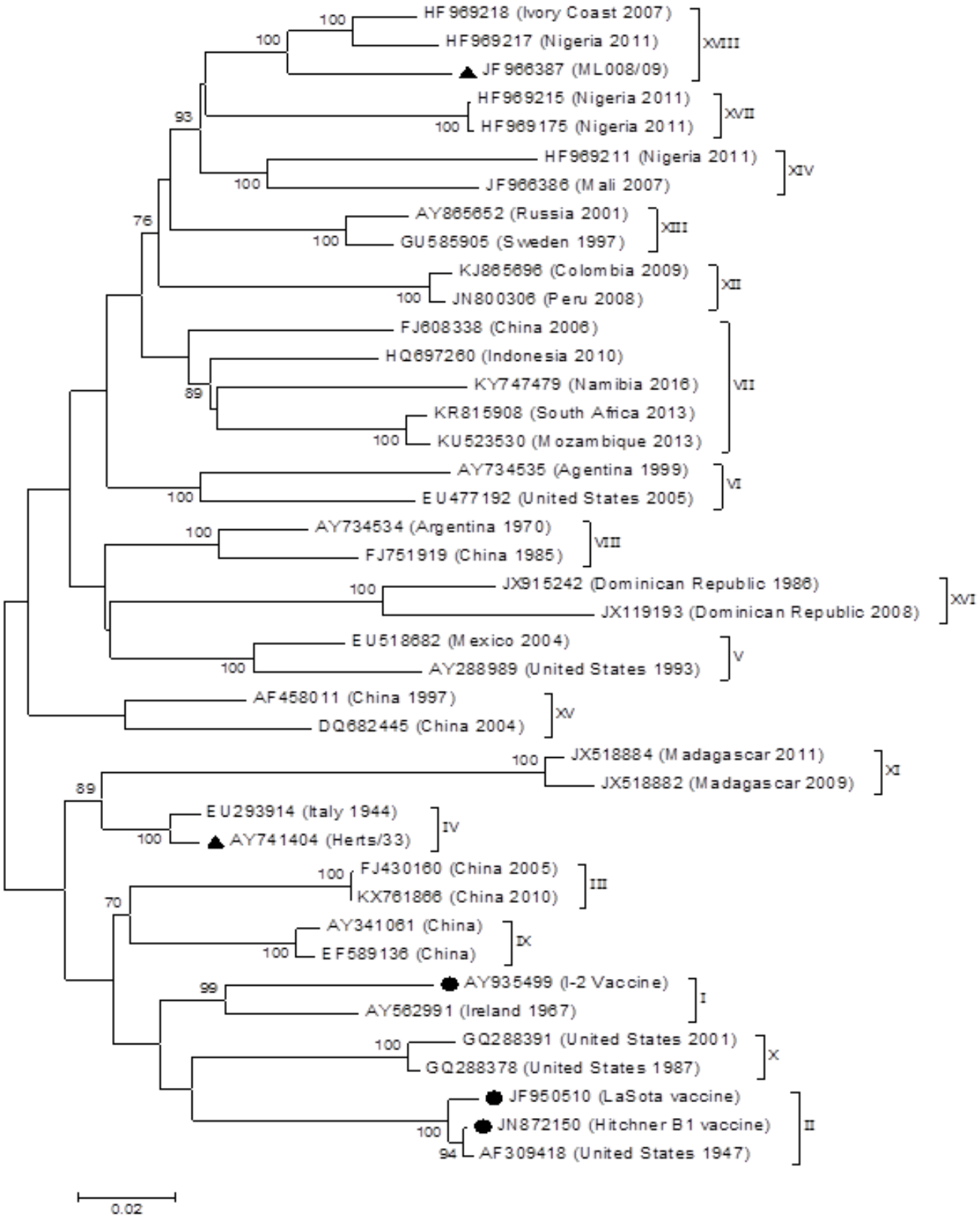

Figure 3. NJ analysis using the MEGA6 software of the full ORF of the fusion (F) protein from representative samples of APMV-1 available in GenBank. The vaccine and challenge viruses used in this study are labelled with a black circle and a black triangle, respectively. The numbers indicate the bootstrap values calculated from 1000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. The different genotypes are shown as described by Diel et al. (2012).

internationally approved OIE standards, under authorizations set forth by the director of the Central Veterinary Laboratory (CVL), Bamako, Mali (N°00439/LCV). The experiments were approved by the Committee for Ethics in animal experiments (Institut National de Recherche en Santé Publique (INRSP) au Mali) under Decision N°10/12/CE-INRSP.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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REFERENCES

Alexander DJ, Manvell RJ, Parsons G (2006). Newcastle disease virus (strain Herts 33/56) in tissues and organs of chickens infected experimentally. Avian Pathology 35(2):99-101.

Samuel A, Baibaswata N, Anandan P, Sa Xiao, Gilbert LA, Kodzo AA, Richard JW, Mariette FD, Peter LC, Siba KS (2013). Phylogenetic and Pathotypic Characterization of Newcastle Disease Viruses Circulating in West Africa and Efficacy of a Current Vaccine. Journal of Clinical Microbiology 51 (3) 771-781; DOI: 10.1128/JCM.02750-12

Bensink Z, Spradbrow P (1999). Newcastle disease virus strain I-2 a prospective thermostable vaccine for use in developing countries. Veterinary Microbiology 68:131-139.

Brown J, Resurreccion RS, Dickson TG (1990). The relationship between the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. Avian Diseases 34(3):585-7.

Cherif A, Bouslama A, Chakroun C, Turki I, Kaboudi K, Bouzouaia M (2010). Suivi sérologique de la vaccination contre les principales viroses aviaires dans les élevages de reproducteurs en Tunisie. Revue d’élevage et de médecine vétérinaire des pays tropicaux 63(1-2):5-11.

Cvelic-Cabrilo V, Mazija H, Bidin Z, Ragland WL (1992). Correlation of haemagglutination inhibition and enzyme-linked immunosorbent assays for antibodies to Newcastle disease virus. Avian Pathology 21(3):509-12

de Leeuw OS, Koch G, Hartog L, Ravenshorst N, Peeters Ben PH (2005). Virulence of Newccastledisease virus determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin-neuraminidaseprotein. Journal of General Virology 86:1759-1769.

Diel DG, da Silva LH, Liu H, Wang Z, Miller PJ, Afonso CL (2012). Genetic diversity of avian paramyxovirus type 1: proposal for a unified nomenclature and classification system of Newcastle disease virus genotypes. Infection, Genetics and Evolution 12:1770-1779.

Dimitrov KM, Lee Dong-Hun, Williams-Coplin Dawn, Olivier Timothy L, Miller Patti J, Afonso Claudia L (2016). Newcastle disease viruse causing recent outbreaks worldwide show unexpectedly high genetic similarity to historical virulent isolates from the 1940s. Journal of clinical Microbiology 54(5):1228-1235.

Dimitrov KM, Afonso CL, Yu Q, Miller PJ (2017). Newcastle disease vaccines-A solved problem or a continuous challenge? Veterinary Microbiology 206:126-136.

Dortmans JC, Venema-Kemper S, Peeters BP, Koch G (2014). Field vaccinated chickens with low antibody titers show equally insufficient protection against matching and non-matching genotypes of virulent Newcastle disease virus. Veterinary Microbiology 172(1-2):100-107.

Dortmans Jos CFM, Peeters Ben PH, Koch Guus (2012). Newcastle disease virus outbreaks: vaccine mismatch or inadequate application. Veterinary Microbiology 160:17-22.

Ezema WS, Okoye JOA, Nwanta JA (2009). LaSota vaccination may not protect against the lesions of velogenic Newcastle disease virus against the lesions of velogenic Newcastle disease in chickens. Tropical Animal. Health Productivity 41:477-484.

Gallili GE, Ben-Nathan D (1998). Newcastle disease vaccines. Biotechnology Advances 16 (2):343-366

Illango J, Olaho-Mukani W, Mukiibi-Muka G, Abila PP, Etoori A (2005). Immunogenicity of a locally-produced Newcastle Disease I-2 thermostable vaccine in chickens in Uganda. Tropical Animal Health and Production, Kluwer Academic Publishers 37:25-31

Maminiaina OF, Gil P, Briand FX, Albina E, Keita D, Andriamanivo HR, Chevalier V, Lancelot R, Martinez D, Rakotondravao R, Rajaonarison JJ, Koko M, Andriantsimahavandy AA, Jestin V, Servan de Almeida R (2010). Newcastle disease virus in Madagascar: identification of an original genotype possibly deriving from a died out ancestor of genotype IV. PLoS ONE 5(11):e13987.

Miller CL, Attrache JE, Dorsey KM, Courtney SC, Guo Z, Kapczynski DR, Afonso PJ (2013). Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. Development and Comparative Immunology 41:505-513.

Miller PJ, King Daniel J, Afonso Claudio L, Suarez David L (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent chamllenge. Vaccine 25:7238-7246.

Nakamura K, Ito M, Nakamura T, Yamamoto Y, Yamada M, Mase M, Imai K (2014). Pathogenesis of Newcastle disease in vaccinated chickens: Pathogenicity of isolated virus and vaccine effect on challenge of its virus. Journal of Veterinary Medical Science *76(1):31-36*.

OIE (2012). Newcastle disease, chapter 2.3.14. In OIE terrestrial Manual 2012: Manual2012 of diagnostic tests and vaccines for terrestrial animals (Paris: World organization for Animal Health), 576-589. Available online at: http://www.oie.int/fileadmin/home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf

de Almeida RS, Saliha H, Patricia G, François-Xavier B, Sophie M, Nicolas G, Julien C, Véronique C, Gilles B, Abdallah T, Colette G, Olivier FM, Samia G, Marthin D, Kassim S, Ould El MB, Abbas D, Hassen C, Flavie G, Peter T, Dominique M, Véronique J, Emmanuel A (2013). New Avian Paramyxoviruses Type I Strains Identified in Africa Provide New Outcomes for Phylogeny Reconstruction and Genotype Classification. PLoS ONE 8:10, e76413

Roohani K, Tan Sheau Wei, Yeap SK, Ideris A, Bejo MH, Omar AR (2015). Characterization of genotype VII Newcastle disease virus isolated from NDV vaccinated chickens and the efficacy of LaSota and recombinant genotype VII vaccines against -challenge with velogenic NDV. Journal of Veterinary Science 16(4):447-457.

Susta L, Jones MEB, Cattoli G, Cardenas-Garcia S, Miller PJ, Brown CC, Afonso CL (2015). Pathologic characterization of genotypes XIV and XVII Newcastle Disease viruses and efficacy pathogen-free birds. Veterinary Pathology 52(1):120-131

Wajid A, Basharat A, Bibi T, Rehmani SF (2018). Comparison of protection and viral shedding following vaccination with Newcastle disease virus strains of different genotypes used in vaccine formulation. Tropical Animal Health and Production May 4.

Young M, Alders R, Grimes S, Spradbrow P, Dias P, da Silva A et Lobo Q (2012). Le Contrôle de la Maladie de Newcastle dans l'aviculture villageoise: un manuel de laboratoire. *2e éd. Monographie ACIAR N° 87a.* Centre Australien International pour la Recherche Agricole: Canberra. ISSN 1031-8194.

SUPPLEMENTARY MATERIAL

Table S1. Characteristics of challenge strains.

Strains	ML008/2009*	Herts/33**
Challenge titer (EID50/ml)	106	106
IPIC	1.8	1.9
Route of challenge	intramuscular	intramuscular

* Strain for trial I; ** Strain for trial II.

Supplementary material: Statistical analysis tables

Table S2: Statistical analysis of the effect of vaccines and protocols on mortality caused by challenge strains.

Observations	111
F (2, 108)	93.57
Prob>F	0.0000
R squared	0.634

Dead	Coefficient	Standard error	t	P	95% Confidence interval	
Group	0.7	0.06	11.45	0.000	0.58	0.82
Vaccine	- 1	0.07	-12.95	0.000	-1.15	-0.85
Constant	- 0.7	0.09	-7.33	0.000	-0.88	-0.51

Table S3. Statistical analysis of mortality in groups after challenge.

Observations	111
F (4, 106)	109.29
Prob>F	0.0000
R squared	0.80

Group	Coefficient	Standard error	t	P>t	95% Confidence interval	
2	0.94	0.05	17.53	0.000	0.83	1.04
3	0.04	0.05	0.67	0.50	-0.07	0.14
4	-0.06	0.05	-1.21	0.23	-0.17	0.04
5	-0.06	0.05	-1.21	0.23	-0.17	0.04
Constant	0.06	0.03	1.93	0.05	-0.001	0.13

Table S4. Results of the statistical analysis of the effect of vaccines on mortality from the viral test virus.

Observations	111
F (3, 107)	12.44
Prob>F	0,0000
R squared	0.26

Vaccines	Coefficient	Standard error	t	P>t	95% Confidence interval	
1	-0.39	0.09	-4.41	0.000	-0.57	-0.22
2	-0.39	0.09	-4.41	0.000	-0.57	-0.22
3	-0.39	0.09	-4.41	0.000	-0.57	-0.22
Constant	0.39	0.05	8.31	0.000	0.30	0.49

Result of analysis of the F gene cleavage site of challenge virus Herts/33 after excretion

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                                100      110        120
(1) IV      Herts/33            RRIQESVTTSG GRRQRRFIGA II
(2) IV     H367T (Group 3)      RRIQESVTTSG GRRQRRFIGA II
(3) IV      H375T (Group 5)     RRIQESVTTSG GRRQRRFIGA II
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Figure S1. Alignment of sequences from viral excretion after the challenge. (1) Reference sequence of Herts/33; (2) Sequence of Herts/33 after excretion in group 3; (3) Sequence of Herts/33 after excretion in group 5.