

Reviewer comment	Reply
Reviewed by 00506467	
<p>The manuscript of Hassanin &amp; Abdel-Moneim describes typing results of various components of Egyptian H5N1 virus (A/chicken/Egypt/F10/2009) an escape mutant to neutralizing antibodies induced by vaccination. However, neither in the present work nor in preceding papers of the authors I found results of a plaque reduction neutralizing test. This test would be necessary to prove that the virus analyzed in the present study indeed is an escape mutant. Therefore, it would be better to exclude this term from the heading and the abstract.</p>	<p>Thank you for your comment, however, many previous studies based on each other can explain the use of the escape mutant in the title and closely related or identical strains were proved to be escape mutant :</p> <p>Grund C, Abdelwhab el-SM, Arafa AS, Ziller M, Hassan MK, Aly MM, Hafez HM, Harder TC, Beer M. Highly pathogenic avian influenza virus H5N1 from Egypt escapes vaccine-induced immunity but confers clinical protection against a heterologous clade 2.2.1 Egyptian isolate. <i>Vaccine</i>. 2011 29(33):5567-73.</p> <p>Terregino C, Toffan A, Cilloni F, Monne I, Bertoli E, Castellanos L, Amarin N, Mancin M, Capua I: Evaluation of the protection induced by avian influenza vaccines containing a 1994 Mexican H5N2 LPAI seed strain against a 2008 Egyptian H5N1 HPAI virus belonging to clade 2.2.1 by means of serological and in vivo tests. <i>Avian Pathol</i> 2010, 39:215-222</p> <p>Abdelwhab el-SM, Erfan AM, Grund C, Ziller M, Arafa AS, Beer M, Aly MM, Hafez HM, Harder TC. Simultaneous detection and differentiation by multiplex real time RT-PCR of highly pathogenic avian influenza subtype H5N1 classic (clade 2.2.1 proper) and escape mutant (clade 2.2.1 variant) lineages in Egypt. <i>Virology</i> 2010 Oct 7;7:260.</p> <p>Arafa A, Suarez DL, Hassan MK, Aly MM: Phylogenetic analysis of HA and NA genes of HPAI-H5N1 Egyptian strains isolated from 2006 to 2008 indicates heterogeneity with multiple distinct sublineages. <i>Avian Dis</i> 2010, 54:345-349.</p> <p>Balish AL, Davis T, Saad MD, El-Sayed N, Esmat H, Tjaden JA, Earhart KC, Ahmed LE, Abd El-Halem M, Hakem M, Ali AM, Nassif SA, El-Ebiary EA, Taha M, Aly MM, Arafa A, O'Neill E, Xiyan X, Cox NJ, Donis RO, Klimov AI:</p>

	<p>Antigenic and genetic diversity of highly pathogenic avian influenza A (H5N1) viruses isolated in Egypt. <i>Avian Dis</i> 2010, 54:329-334</p> <p>Kim J, Kayali G, Walker D, Forrest HL, Ellebedy AH, Griffin YS, Rubrum A, Bahgat MM, Kutkatd MA, Ali MAA, Aldridge JR, Negovetich NJ, Krauss S, Webby RJ, Webster RG: Puzzling inefficiency of H5N1 influenza vaccines in Egyptian poultry. <i>Proc Natl Acad Sci</i> 2010, 107:11044-9.</p> <p>Abdel-Moneim AS, Afifi MA, El-Kady MF. Genetic drift evolution under vaccination pressure among H5N1 Egyptian isolates. <i>Virology</i> 2011 8:283.</p>
<p>While the “Introduction” section is well written and easy to understand parts of the “Results and Discussion” section should be rephrased. There are some sentences I do not understand:</p> <p>Page 5:  “‘All the screened alleles of the F10: PB2, PB1, PA, NP, NA, M, and NS alleles are Eurasia in origin: K, G, D, F, 1J, F, and 1E respectively[16].”</p>	Fixed: Lines 116-117
<p>Page 6: “However, this substitution possesses marginally significant to virulence in mammals [22].”</p>	Fixed: Lines 141-142
<p>Page 6: The authors state that some residues are virulent or nonvirulent. Is this statement true? I guess the residues are associated with high or low pathogenicity?</p>	Virulent and non virulent amino acid residues refer to the ability of the virus to replicate in mammals as determined by Lycett et al
<p>Abstract: Evolution phylogenetic trees  Suggestion: Evolution phylogenetic trees</p>	Fixed : Line 30
<p>Page 5: ... amino-acid R at position 110... Suggestion: ...amino-acid arginine (R) at position 110</p>	Fixed: Lines 120-121
<p>Two spellings are used within the document: amino-acids / amino acids  Please use consistent spelling.</p>	Fixed (Multiple)
<p>Page 5: The six internal genes (PB2, PB1, PA, NP, M and NS) of A/chicken/Egypt/F10/2009, and most of Egyptian H5N1 strains amino showed avian like amino acid signatures (Table 2). Suggestion: The six internal genes</p>	Fixed : Lines 126-127

<p>(PB2, PB1, PA, NP, M and NS) of A/chicken/Egypt/F10/2009, and most of Egyptian H5N1 strains amino-acids showed avian like amino-acid signatures (Table 2).</p>	
<p>Page 6: ... were detected in the avian H5N1 strains in PB2 (K627) M2 (S64, P69), and NS1 (S42, E92/97) ... Suggestion: ... were detected in the avian H5N1 strains in PB2 (K627), M2 (S64, P69), and NS1 (S42, E92/97) ...</p>	Fixed: Line 134-135
<p>Page 6: ... residues in PB2 (K627) M2 (P69), and NS1 (S42, E92/97). Suggestion: ... residues in PB2 (K627), M2 (P69), and NS1 (S42, E92/97).</p>	Fixed: Lines 136-137
<p>Page 6: Viruses showed (E627) exhibited cold sensitivity whereas viruses with (K627) displayed higher activity of the polymerase complex during viral replication at a lower temperature[26] Suggestion: Viruses showing E627 exhibited cold sensitivity whereas viruses with K627 displayed higher activity of the polymerase complex during viral replication at a lower temperature[26]</p>	Fixed: Lines 144-145
<p>Page 6: Efficient virus replication may explain the wide host range of subtype H5N1 strains and their the high virulence [26]. PB2 of all Egyptian strains including avian, mammalian isolates possessed K627 (Table 3). Suggestion: Efficient virus replication may explain the wide host range of subtype H5N1 strains and their high virulence [26]. PB2 of all Egyptian strains including avian, mammalian isolates possessed K627 (Table 3).</p>	Fixed: Lines 146-148
<p>Reviewed by 00504040</p>	
<p>This study is suitable for publication in WJV after giving polish of the English contents. e.g. (1)In Egypt vaccination of poultry with inactivated vaccine preparations is adopted to combat the H5N1 however, vaccination of household poultry was suspended in the mid of 2009 due limited impact on H5N1 incidence[8]. (2) most of Egyptian H5N1 strains amino showed avian like amino acid signatures</p>	<p>Fixed</p> <p>Lines 52-53</p> <p>Lines 126-127</p>

<p>(3) Viruses showed (E627) exhibited cold sensitivity whereas viruses with (K627) displayed higher activity of the polymerase complex during viral replication at a lower temperature[26] (4).....</p>	<p>Lines 144-145</p>
<p>Reviewed by 00483991</p>	
<p>Do the author's have function evidence that this strain is an escape mutant?</p>	<p>Thank you for your comment, however, many previous studies based on each other can explain the use of the escape mutant in the title and closely related or identical strains were proved to be escape mutant :</p> <p>Grund C, Abdelwhab el-SM, Arafa AS, Ziller M, Hassan MK, Aly MM, Hafez HM, Harder TC, Beer M. Highly pathogenic avian influenza virus H5N1 from Egypt escapes vaccine-induced immunity but confers clinical protection against a heterologous clade 2.2.1 Egyptian isolate. <i>Vaccine</i>. 2011 29(33):5567-73.</p> <p>Terregino C, Toffan A, Cilloni F, Monne I, Bertoli E, Castellanos L, Amarin N, Mancin M, Capua I: Evaluation of the protection induced by avian influenza vaccines containing a 1994 Mexican H5N2 LPAI seed strain against a 2008 Egyptian H5N1 HPAI virus belonging to clade 2.2.1 by means of serological and in vivo tests. <i>Avian Pathol</i> 2010, 39:215-222</p> <p>Abdelwhab el-SM, Erfan AM, Grund C, Ziller M, Arafa AS, Beer M, Aly MM, Hafez HM, Harder TC. Simultaneous detection and differentiation by multiplex real time RT-PCR of highly pathogenic avian influenza subtype H5N1 classic (clade 2.2.1 proper) and escape mutant (clade 2.2.1 variant) lineages in Egypt. <i>Virol J</i>. 2010 Oct 7;7:260.</p> <p>Arafa A, Suarez DL, Hassan MK, Aly MM: Phylogenetic analysis of HA and NA genes of HPAI-H5N1 Egyptian strains isolated from 2006 to 2008 indicates heterogeneity with multiple distinct sublineages. <i>Avian Dis</i> 2010, 54:345-349.</p> <p>Balish AL, Davis T, Saad MD, El-Sayed</p>

	<p>N, Esmat H, Tjaden JA, Earhart KC, Ahmed LE, Abd El-Halem M, Hakem M, Ali AM, Nassif SA, El-Ebiary EA, Taha M, Aly MM, Arafa A, O'Neill E, Xiyan X, Cox NJ, Donis RO, Klimov AI: Antigenic and genetic diversity of highly pathogenic avian influenza A (H5N1) viruses isolated in Egypt. <i>Avian Dis</i> 2010, 54:329-334</p> <p>Kim J, Kayali G, Walker D, Forrest HL, Ellebedy AH, Griffin YS, Rubrum A, Bahgat MM, Kutkatd MA, Ali MAA, Aldridge JR, Negovetich NJ, Krauss S, Webby RJ, Webster RG: Puzzling inefficiency of H5N1 influenza vaccines in Egyptian poultry. <i>Proc Natl Acad Sci</i> 2010, 107:11044-9.</p> <p>Abdel-Moneim AS, Afifi MA, El-Kady MF. Genetic drift evolution under vaccination pressure among H5N1 Egyptian isolates. <i>Viol J.</i> 2011 8:283.</p>
Abstract: 1. Spelling error “phylogenetic trees”	Fixed: Line 30
Introduction: 1. This sentence needs to be restructured “Interspecies transmission can be devastating with all catastrophic consequences”	Done: Lines 44-45
2. “with 62 fatal cases” annually or total recorded?	Fixed: Line 51
Results and Discussion: 1. “Analysis of the NA gene revealed the presence of the 20-amino acid deletion” Would this deletion not be lethal for the virus? Please discuss.	<p>Lines: 118-120</p> <p>A deletion in the stalk of the NA, a feature that is frequently seen during the process of adaptation of influenza viruses from wild aquatic birds to poultry that favours viral replication and enhances pathogenesis in chickens [Munier S, Larcher T, Cormier-Aline F, Soubieux D, Su B, Guigand L, Labrosse B, Cherel Y, Quéré P, Marc D, Naffakh N. A genetically engineered waterfowl influenza virus with a deletion in the stalk of the neuraminidase has increased virulence for chickens. <i>J Virol.</i> 2010 Jan;84(2):940-52.].</p>
2. “In conclusion, the genetic characteristics of the H5N1 virus isolates from chicken in Egypt provided evidences to high possibility of inter-	This conclusion was explained in the results and discussion section due to the presence of some virulent residues and amino acid substitutions related to

<p>species transmission.” This is obviously a significant claim. I was wondering how a “high possibility” is deduced? What % virus homology is required for bird/human transmission for instance?</p>	<p>mammalian virulence in the examined stain.</p>
<p>Figures: 1.Fig1 is misshapen</p>	<p>According to the WJV, Figure is enclosed in the text so when it will be in a final form, they will require to submit it as separate JPG file and it will greatly enhance the Figure.</p>