

Marek's Disease: rapid progress in research with unclear biological implementations

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Summary. – Here we would like to provide a brief overview of the modern history of Marek's disease (MD) research with a focus on the most recent developments in experimental work and we will try to sum up their impact on the understanding of the biological properties of Marek's disease type 1 (MDV-1), the only representative of the *Mardivirus* genus causing fatal lymphoproliferative disease in poultry. We will also compare MDV-1 with other serologically-related poultry herpesviruses, Marek's disease virus type 2 (MDV-2) and herpesvirus of turkeys (HVT). Although MD was first described at the beginning of the last century, proper characterization of its biological impact on poultry production and utilization of molecular biology methods for detailed characterization of causative agent MDV-1 were introduced only in recent decades. However, many characteristics of MD infection, pathogenesis and vaccine protection mechanisms remain unclarified, though novel methods bring a challenge for better understanding of these unanswered questions.

Keywords: Marek's disease; Marek's disease virus; lymphoproliferative neoplastic disease; Meq oncoprotein

Contents:

1. Overview of Marek's disease history
 - 1.1 The importance of the 1960's and 70's in the introduction of modern techniques to the study of the biology of Marek's disease
 2. Molecular pathogenesis of Marek's disease
 - 2.1 Meq protein (MDV EcoRI-Q oncoprotein)
 - 2.2 microRNAs (miRNAs) encoded by MDV-1
 - 2.3 Transcripts expressed in the repeat regions of the MDV-1 genome
 3. Vaccines against Marek's disease and their function
 4. New genetic approaches to the study of Marek's disease and related viruses

1. Overview of Marek's disease history

The disease was primarily described as polyneuritis in chickens (Marek, 1907), and decades later named by its founder, Professor of the Department of Veterinary Medicine at the Hungarian Veterinary School, Jozsef Marek (from Horná Streda and currently residing in the Slovak Republic). The primary discovery however brought no deep interest in the scientific and poultry production fields as paralysis of the legs and wings was rather uncommon at that time. However awareness began to increase with the intensification of the poultry production business in the 1950's and 60's, when more avian pathogens began to pose a threat to the business itself with great losses in production. At this time, MD also became a serious problem when transformed lymphocytes began to be more invasive not just to the nervous system but also causing lymphoid tumours in visceral organs, mainly the spleen, Bursa Fabricius, thymus, liver, kidney, ovary, proventriculus and later, with more pathogenic strains in the heart and muscles also.

In the first half of the last century, there was confusion in relation to the aetiological causative agent of the lethal disease as neoplastic conditions of homeopathic system of

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Abbreviation: BAC = bacterial artificial chromosome; HSV-1 = herpes simplex virus 1; HVT = herpesvirus of turkeys serotype 3; MeHV-1 = Meleagrid herpesvirus 1; MD = Marek's Disease; MDV-1 and 2 = Marek's disease virus type 1 and 2, GaHV-2 and 3 = Gallid herpesvirus 2 and 3; Meq = MDV EcoRI-Q oncoprotein; miRNA = microRNA

domestic chickens were also described (Ellermann, 1921). In 1961 it was proposed by Biggs (1961) to differentiate in classification between lymphoid leucosis caused by retroviruses and fowl paralysis, later called MD.

The real breakthrough came in the late 1960's when the aetiological agent of MD was identified and characterized and new approaches to control the disease were introduced.

1.1 The importance of the 1960's and 70's in the introduction of modern techniques to the study of the biology of Marek's disease

Development of a cell culture system for propagation of MD causative agent using infectious blood or tumour tissues resulted in visible cytopathic effect in *in vitro* cultured chicken kidney cells (Churchill and Biggs, 1967) or duck embryo fibroblasts (Nazerian *et al.*, 1968). These pioneering findings resulted in an increase in basic and molecular biological studies on MD and its causative agent Marek's disease virus type 1 (MDV-1) currently classified as Gallid herpesvirus 2 (GaHV-2). MDV-1 was found in both cases as strongly cell-associated in *in vitro* cell culture. The source of fully infectious, cell-free MDV-1 virus has been described only in chicken feather follicles (Calnek *et al.*, 1970).

Soon after identification of the causative agent of MD, the first live attenuated vaccines against the disease were prepared by serial *in vitro* passage of mild HPRS-16 strain of MDV-1 (Churchill *et al.*, 1969). This vaccine was soon replaced by serologically related herpesvirus of turkeys (HVT) currently classified as Meleagrid herpesvirus 1 (MeHV-1) which is apathogenic to chickens as well as to turkey (Okazaki *et al.*, 1970; Witter *et al.*, 1970).

The late 1960's till the early 90's brought about a significant breakthrough in molecular biology characterization of MDV-1 and serologically related apathogenic avian herpesviruses, Marek's disease virus type 2 (MDV-2) currently classified as Gallid herpesvirus 3 (GaHV-3) and HVT. Restriction enzyme maps and cloned fragments of their genomes were prepared (Gibbs *et al.*, 1984; Hirai *et al.*, 1979; Ross *et al.*, 1983). Many fragments of MDV-1, MDV-2, and HVT genome were sequenced and found to be homologous to *Alphaherpesviruses* genes with similar co-localization mainly within the unique long (U_L) or unique short (U_S) parts of the respective genomes, e.g. as described in comparison with varicella-zoster virus by Buckmaster *et al.* (1988).

In past, all strains of virus originally designed as Marek's disease virus (MDV) were divided to serotypes 1-3 and also classified as so-called virulent (v), very virulent (vv), or very virulent plus (vv+) strains (Schat *et al.*, 1982; Witter, 1997). Recent nomenclature reclassifies serotype 1 encompassing all pathogenic or oncogenic strains as GaHV-2,

serotype 2 encompassing naturally non-pathogenic strains as GaHV-3, and serotype 3 encompassing non-oncogenic viruses isolated from turkeys as MeHV-1, respectively. They all are members of the *Mardivirus* genus (the *Alphaherpesvirinae* subfamily, the *Herpesviridae* family).

A "boom" in molecular characterization of MDV-1 occurred in the late 1990's and it is the fact that there is still big interest to study the disease.

It is of great importance to keep in mind that MDV-1 is an evolving pathogen. Despite the fact that it is a DNA-encoded virus with lower frequency of mutations compared to RNA viruses, MDV-1 has been shown to be a pathogen which adapts to the environment even under conditions of preventive vaccination strategies. Under these conditions, mild MDV-1 that could have been prevented by HVT, underwent changes that resulted in the occurrence of very virulent MDV-1 strains resistant to HVT vaccination (Schat *et al.*, 1982). Even after the introduction of bivalent MD vaccines based on use of live apathogenic HVT and MDV-2 mixture, viruses of MDV-1 breaking the vaccine protection were isolated vaccine in 1990's (Witter, 1997). Whether extensive use of MD vaccines can drive MDV-1 to its increased virulence is up for discussion (Davison and Nair, 2005). It is clear that serial *in vitro* passage of MDV-1 causes attenuation of the virus with dynamic changes in its genome (Fukuchi *et al.*, 1985; Kopacek *et al.*, 1993; Silva *et al.*, 1985). Although there is still a general feeling that we do not fully understand or have undermined the impact of these genetic changes on the biological properties of different MDV-1 isolates and their passage history, the recent results of some authors are opening new insights into this topic (Spatz *et al.*, 2012).

2. Molecular pathogenesis of Marek's disease

As mentioned above, MD is a highly contagious neoplastic disease mostly affecting chickens but in some cases also turkeys. The main syndromes named under "classical" MD include neurolymphomatosis resulting in nerve paralysis and lymphatous infiltration as well as tumours in visceral organs, muscles and skin. New vv and vv+ MDV strains are even more aggressive, causing death in very young birds (reviewed by Calnek, 2001).

MD is certainly among a category of diseases with complex pathogenesis. These attributes relate mainly to the virulence of relevant MDV-1 strain, genetic background of the infected host and overall management of hygiene in animal houses. Nevertheless, with intensified poultry production, serologically related avian herpesviruses with different impacts on pathogenesis and/or protective potential have been reported and isolated (a summary of these *Mardivirus* genus isolates can be found in Table 1).

Table 1. *Mardivirus* genus viruses characterized by their serotypes and pathotypes

| Standard nomenclature | Virus | | Pathotype/clinical signs | Representative isolates |
|--------------------------------------|----------|----------------------------------|--|---|
| | Serotype | Current classification | | |
| Marek's disease virus type 1 (MDV-1) | 1 | Gallid herpesvirus 2 (GaHV-2) | vv+ MDV, very virulent plus MDV-1 strains early age mortality caused by immunosuppression and failure of protection by bivalent MDV-2 + HVT vaccines | 648A (Witter, 1997; Spatz <i>et al.</i> , 2012) |
| | | | vv MDV, very virulent MDV-1 strains/ very low protection induced by HVT-based vaccines | Md5 (Tulman <i>et al.</i> , 2000); RB-1B (Schat <i>et al.</i> , 1982) |
| | | | v MDV, virulent MDV-1 strains | HPRS-16 (Purchase and Biggs, 1967); JM (Sevoian <i>et al.</i> , 1962) |
| | | | Low pathogenic MDV-1 strains | CVI988/Rispens (Rispens <i>et al.</i> , 1972) |
| Marek's disease virus type 2 (MDV-2) | 2 | Gallid herpesvirus 3 (GaHV-3) | Non-oncogenic MDV-2 serologically related to MDV-1 | SB-1 (Schat and Calnek, 1978) |
| Turkey herpesvirus (HVT) | 3 | Meleagrid herpesvirus 1 (MeHV-1) | Non-pathogenic herpesviruses isolated from turkey, serologically related to MDV viruses. | Fc126 (Okazaki <i>et al.</i> , 1970) |

Thanks to novel approaches in studies of molecular properties of MD, we can predict and conclude the most prominent determinants in MD pathogenesis.

2.1 *Meq* protein (MDV *EcoRI*-Q oncoprotein)

Meq protein encoded by MDV-1 is a member of a Jun/Fos oncogene protein family (Jones *et al.*, 1992). *Meq* is a unique gene encoded by oncogenic strains of MDV-1 without homologous partners present in genomes of MDV-2 and/or HVT. It encodes for a 339 amino-acid protein highly expressed in MDV-induced tumours and lymphoblastoid cell lines. *Meq* protein is the strongest candidate oncoprotein, potentially associated with other factors, encoded by MDV-1.

It should be mentioned that there is also L-*Meq* gene presented in genomes of low pathogenic MDV-1 strains, e.g. CVI988/Rispens that have a 59 amino-acid proline-rich insert in the protein encoded (Lee *et al.*, 2000). As proposed in some works, this might be an explanation as to why low pathogenic MDV-1 strains lack oncogenic potential typical for highly oncogenic MDV-1 viruses. For further analysis of *Meq* protein transformation potential, Rat-2 cells were transformed to over-express *Meq* resulting in the inhibition of apoptosis in the transformed cell line (Liu *et al.*, 1998).

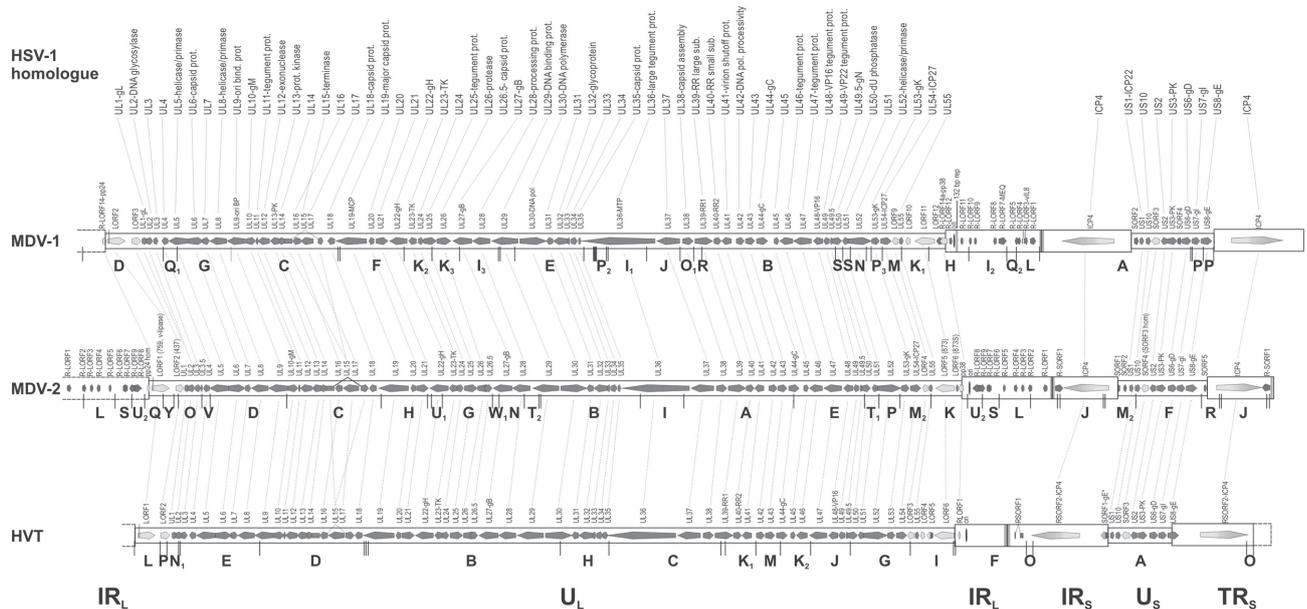
2.2 microRNAs (miRNA) encoded by MDV-1

miRNAs is a family of small RNA molecules that regulate gene expression at its post-transcriptional level.

The majority of large DNA viruses, mostly herpesviruses, encode their own miRNAs. Although the members of the *Mardivirus* genus encode their own set of miRNAs for each virus, there is significant evidence that MDV-1 encodes miRNAs that are related to MDV-1 pathogenesis. Primary studies indicated that these miRNAs flank to the *Meq* oncogene and/or map to latency-associated transcripts that are antisense to the immediate-early ICP4 gene (Burnside, *et al.*, 2006). Stick *et al.* (2013) proposed that gga-miR-21 is upregulated during MDV-1 infection, indicating that *Meq* oncoprotein map to the relevant gga-miR-21 promoter of the *TMEM49* gene. It was found that gga-miR-21 is overexpressed only upon infection with a very virulent RB1-B strain but not during infection with low pathogenic strain of MDV-1, CVI988/Rispens. There exists a great challenge to further explore the phenomenon of miRNAs in MD pathogenesis.

2.3 Transcripts expressed in the repeat regions of MDV-1 genome

pp38 and related transcripts encoded within the region of the *Bam*HI-H restriction fragment of MDV-1 genome linking U_L and terminal repeat long (TR_L) region of the MDV-1 genome were thought to be directly involved in oncogenicity and pathogenesis (Ross *et al.*, 1993). The majority of references report to 132 bp long repeats present within these regions that are multiplied during *in vitro* passage of the infectious virus (Silva and Barnett, 1991; Kopacek *et al.*, 1993). However, it was clearly shown that 132 bp repeats



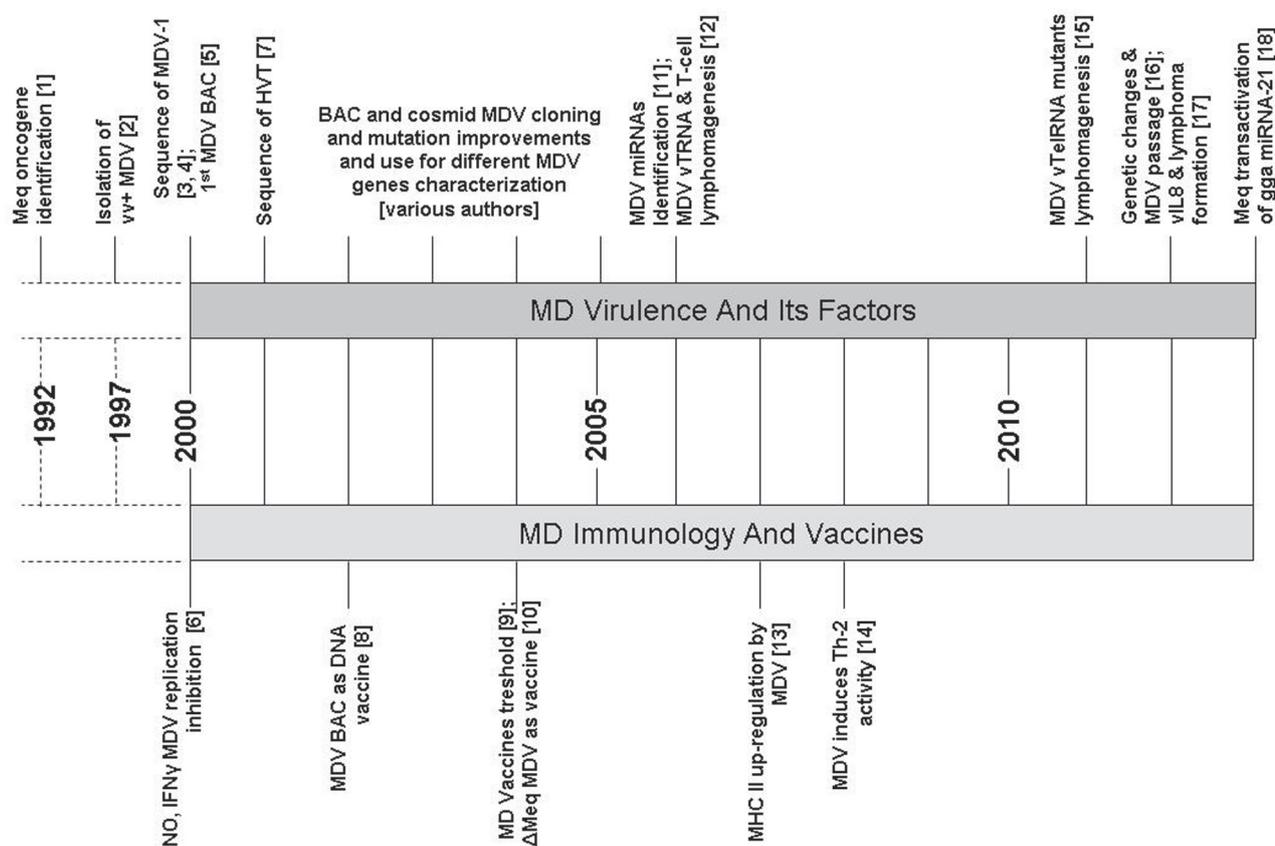


Fig. 2

Overview of selected the most current achievements in Marek's disease research

References in brackets respond to: [1] Jones *et al.*, 1992; [2] Witter 1997; [3] Lee *et al.*, 2000; [4] Tulman *et al.*, 2000; [5] Schumacher *et al.*, 2000; [6] Xing and Schat 2000; [7] Kingham *et al.*, 2001; [8] Tischer *et al.*, 2002; [9] Witter and Kreeger 2004; [10] Lupiani *et al.*, 2004; [11] Burnside *et al.*, 2006; [12] Trapp *et al.*, 2006; [13] Niikura *et al.*, 2007; [14] Heidari *et al.*, 2008; [15] Kaufer *et al.*, 2011; [16] Spatz *et al.*, 2012; [17] Engel *et al.*, 2012; [18] Stik *et al.*, 2013.

4. New genetic approaches to the study of Marek's disease and related viruses

Since the year 2000 new approaches have been taken to the genetic characterization of MDV-1. First of all, a full sequence of virulent MDV-1 (Lee *et al.*, 2000; Tulman *et al.*, 2000) and its serologically related HVT were published (Kingham *et al.*, 2001). Gene layout of individual serologically related avian herpesviruses is graphically outlined in Fig. 1.

The real breakthrough in the study and characterization of MDV-1, MDV-2, and HVT genes and genetic elements came after introduction of bacterial artificial chromosome (BAC) technology (Schumacher *et al.*, 2000; reviewed also in Zelnik, 2003). This novel approach also allowed for the preparation of an experimental DNA vaccine for the protection of chickens against MD (Tischer *et al.*, 2002). The target of this minireview is not to describe all achievements

resulting from the introduction of BAC technology to MD research. However a summary of these accomplishments since the introduction of this technology might be concluded in a separate review.

A brief summary on Marek's disease research with the most important outputs including timetable is provided in Fig.2.

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References

- Biggs PM, Br. Vet. J. 117, 326-334, 1961.
 Buckmaster AE, Scott SD, Sanderson MJ, Bournnell MEG, Ross NLJ, Binns MM, J. Gen. Virol. 69, 2033-2042, 1988. <http://dx.doi.org/10.1099/0022-1317-69-8-2033>

- Burnside J, Bernberg E, Anderson A, Lu C, Meyers BC, Green PJ, Jain N, Isaacs G, Morgan RW, J. Virol. 80, 8778-8786, 2006. Calnek BW, In Hirai K (Ed), Springer-Verlag Berlin, Heidelberg, pp. 32-41, 2001.
- Calnek BW, Adlindger HK, Kahn DE, Avian Dis. 14, 219-233, 1970. <http://dx.doi.org/10.2307/1588466>
- Camp HS, Coussens PM, Silva RF, J. Virol. 65, 6320-6324, 1991.
- Churchill AE, Biggs PM, Nature 215, 528-530, 1967. 2004.
- Churchill AE, Payne LN, Chubb RC, Nature 221, 744-747, 1969. <http://dx.doi.org/10.1038/221744a0>
- Davison F, Nair V, Expert Rev. Vacc. 4, 1-12, 2004.
- Ellermann V, Gyldendal, London, 1921.
- Engel AT, Selvaraj RK, Kamil JP, Osterrieder N, Kaufer BB, J. Virol. 86, 8536-8545, 2012. <http://dx.doi.org/10.1128/JVI.00556-12>
- Fukuchi K, Tanaka A, Schierman LW, Witter RL, Nonoyama M, Proc. Natl. Acad. Sci. USA 82, 751-754, 1985. <http://dx.doi.org/10.1073/pnas.82.3.751>
- Gibbs CP, Nazerian K, Velicer LF, Kung H-J, (1984): Proc. Natl. Acad. Sci. USA 81, 3365-3369, 1984. <http://dx.doi.org/10.1073/pnas.81.11.3365>
- Heidari M, Zhang HM, Sharif S, Viral. Immunol. 21, 203-214, 2008
- Hirai K, Ikuta K, Kato S, J. Gen. Virol. 45, 119-131, 1979. <http://dx.doi.org/10.1099/0022-1317-45-1-119>
- Jones D, Lee L, Liu J-L, Kung H-J, Tillotson JK, Proc. Natl. Acad. Sci. USA 89, 4042-45046, 1992. <http://dx.doi.org/10.1073/pnas.89.9.4042>
- Kaufer BB, Arndt S, Trapp S, Osterrieder N, Jarosinski KW, PLoS Pathogens 7, e1002333, 2011. <http://dx.doi.org/10.1371/journal.ppat.1002333>
- Kingham BW, Zelnik V, Kopacek J, Majerciak V, Ney E, Schmidt CJ, J. Gen. Virol. 82, 1123-1135, 2001.
- Kopacek J, Ross LJN, Zelnik V, Pastorek J, Acta Virol. 37, 191-195, 1993.
- Lee SI, Takaqi M, Ohashi K, Suquimoto C, Onuma M, J. Vet. Med. Sci. 62, 287-292, 2000. <http://dx.doi.org/10.1292/jvms.62.287>
- Lee FL, Wu P, Sui D, Ren D, Kamil J, Kung H-J, Witter RL, Proc. Natl. Acad. Sci. USA 97, 6091-6096, 2000. <http://dx.doi.org/10.1073/pnas.97.11.6091>
- Liu J-L, Ye Y, Lee LF, Kung H-J, J. Virol. 72, 388-395, 1998.
- Lupiani B, Lee LF, Cui X, Gimeno I, Anderson A, Morgan RW, Silva RF, Witter RL, Kung HJ, Reddy SM, Proc. Natl. Acad. Sci. USA 101, 11815-20, 2004.
- Marek J, Deutsche Tierarztzeitung Wschr. 15, 417-421, 1907.
- Nazerian K, Solomon JJ, Witter RL, Burmester BR, Proc. Soc. Exp. Biol. Med. 127, 177-182, 1968.
- Niikura M, Kim T, Hunt HD, Burnside J, Morgan RW, Dodgson JB, Cheng HH, Virology 359, 212-219, 2007.
- Okazaki W, Purchase HG, Burmester BR, Avian Dis. 14, 413-429, 1970. <http://dx.doi.org/10.2307/1588488>
- Purchase HG, Biggs PM (1967): Res. Vet. Sci. 8, 440-449, 1967.
- Rispens BH, von Vloten H, Mastenbroek N, Maas, HJL, Schat KA, Avian Dis. 16, 108-125, 1972. <http://dx.doi.org/10.2307/1588905>
- Ross N, Binns MM, Sanderson M, Schat KA, Virus Genes 7, 33-51, 1993. <http://dx.doi.org/10.1007/BF01702347>
- Ross LJN, Milne B, Biggs PM, J. Gen. Virol. 64, 2785-2790, 1983. <http://dx.doi.org/10.1099/0022-1317-64-12-2785>
- Schat KA, Calnek BW, (1978): J. Nat. Cancer Inst. 60, 1075-1082, 1978.
- Schat KA, Calnek BW, Fabricant J, Avian Pathol. 11, 593-605, 1982. <http://dx.doi.org/10.1080/03079458208436134>
- Schat KA, Markowski-Grismund, In Hirai K (Ed), Springer-Verlag, Berlin, Heidelberg, New York, pp. 91-120, 2001.
- Schumacher D, Tischer BK, Fuchs W, Osterrieder N, J. Virol. 74, 11088-11098, 2000. <http://dx.doi.org/10.1128/JVI.74.23.11088-11098.2000>
- Silva RF, Barnett JC, Avian Dis. 35, 487-495, 1991. <http://dx.doi.org/10.2307/1591212>
- Silva RF, Reddy SM, Lupiani B, J. Virol. 78, 733-740, 2004. <http://dx.doi.org/10.1128/JVI.78.2.733-740.2004>
- Silva RF, Witter RL, J. Virol. 54, 690-696, 1985.
- Sharma JM, Burmester BR, Avian Dis. 27, 453-463, 1983. <http://dx.doi.org/10.2307/1590171>
- Spatz SJ, Volkening JD, Gimeno IM, Heidari M, Witter RL, Virus Genes 45, 526-536, 2012. <http://dx.doi.org/10.1007/s11262-012-0792-z>
- Stick G, Dambrine G, Pfeffer S, Rassachaert D, J. Virol. 87, 80-93, 2013. <http://dx.doi.org/10.1128/JVI.02449-12>
- Trapp S, Parcells MS, Kamil JP, Schumacher D, Tischer BK, Kumar PM, Nair VK, Osterrieder N, J. Exp. Med. 203, 1307-1317, 2006. <http://dx.doi.org/10.1084/jem.20052240>
- Tischer BK, Schumacher D, Beer M, Beyer J, Teifke JP, Osterrieder K, Wink K, Zelnik V, Fehler F, Osterrieder N, J. Gen. Virol. 83, 2367-2376, 2002.
- Tulman ER, Afonso CL, Lu Z, Zsak L, Rock DL, Kutish GF, J. Virol. 74, 7980-7988, 2000. <http://dx.doi.org/10.1128/JVI.74.17.7980-7988.2000>
- Witter RL, Avian Dis. 41, 149-163, 1997. <http://dx.doi.org/10.2307/1592455>
- Witter RL, Kreager KS, Avian Dis. 48, 768-782, 2004. <http://www.bioone.org/doi/abs/10.1637/7203-050304R-aff2#aff2> <http://dx.doi.org/10.1637/7203-050304R>
- Witter RL, Nazerian K, Purchase HG, Burgoyne GH, Am. J. Vet. Res. 31, 525-538, 1970.
- Xing Z, Schat KA, Immunology 100, 70-6, 2000.
- Zelnik V, Avian Pathol. 32, 323-333, 2003. <http://dx.doi.org/10.1080/0307945031000121068>
- Zelnik V, Harlin O, Fehler F, Kaspers B, Göbel TW, Nair VK, Osterrieder N, J. Vet. Med., Ser. B 51, 61-67, 2004.