UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

CARACTERIZAÇÃO DO VIROMA FECAL DE FRANGOS DE CORTE SAUDÁVEIS E AFETADOS PELA SÍNDROME DA MÁ ABSORÇÃO

DIANE ALVES DE LIMA

Porto Alegre
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Tese apresentada como requisito parcial para obtenção do título de doutora em Ciências Veterinárias na área de Microbiologia Veterinária – Virologia.

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Coorientador: Dr. Samuel Paulo Cibulski

Porto Alegre

2018

CIP - Catalogação na Publicação

Lima, Diane Alves de
CARACTERIZAÇÃO DO VIROMA FECAL DE FRANGOS DE CORTE SAUDÁVEIS E AFETADOS PELA SÍNDROME DA MÁ ABSORÇÃO / Diane Alves de Lima. -- 2018.
116 f.
Orientador: Paulo Michel Roehe.

Coorientador: Samuel Paulo Cibulski.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Veterinária, Programa de Pós-Graduação em Ciências Veterinárias, Porto Alegre, BR-RS, 2018.

1. viroma fecal. 2. frangos de corte. 3. distúrbios entéricos. 4. sequenciamento de alto desempenho. I. Roehe, Paulo Michel, orient. II. Cibulski, Samuel Paulo, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

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Aprovada em 28 FEV 2018

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AGRADECIMENTOS

Agradeço à minha mãe Erci, pelo exemplo, apoio e dedicação em me auxiliar sempre que possível. Ao meu marido, Moisés, que esteve ao meu lado em todos os momentos, demonstrando paciência, cumplicidade e carinho.

Ao meu orientador Paulo Roehe pelos ensinamentos repassados e por toda ajuda na construção deste trabalho. Sobretudo agradeço a confiança em mim depositada e a oportunidade de crescimento.

Ao meu coorientador Samuel pela imensa paciência, pelos ensinamentos e por toda ajuda até aqui. Agradeço também pela amizade e palavras de incentivo.

A todos os colegas do laboratório de virologia do IPVDF e do LabVir/UFRGS pelo apoio, pelo auxílio nos trabalhos de bancada, nas coletas, nas análises, bem como nas discussões.

Agradeço à CAPES pela concessão da bolsa e aos demais órgãos de fomentos à pesquisa, FINEP e CNPq, pelo apoio financeiro durante o desenvolvimento do projeto.

Muito obrigada a todos!

RESUMO

A síndrome da má absorção (SMA) é uma doença de aves jovens caracterizada por retardo no crescimento, desenvolvimento defeituoso das penas e diarreia, resultando em relevante impacto econômico. Estudos prévios têm investigado a participação de uma série de agentes virais na etiologia da SMA. No entanto, o conhecimento limitado da comunidade viral presente no intestino das aves dificulta a identificação precisa do(s) agente(s) causal(is). Nesse sentido, o presente estudo foi conduzido visando ampliar o conhecimento sobre o viroma entérico de frangos de corte jovens (3-5 semanas) e seu envolvimento com a ocorrência da SMA. Para tanto, em quatro granjas, foram feitas coletas de conteúdo intestinal de frangos clinicamente saudáveis. DNA e RNA virais foram extraídos, sequenciados e comparados com dados previamente disponíveis através de análises metagenômicas. Genomas representantivos de uma ampla diversidade de reconhecidas famílias virais (*Adenoviridae*, *Caliciviridae*, *Circoviridae*, *Parvoviridae*, *Picobirnaviridae*, *Picornaviridae* e *Reoviridae*) foram identificados. Igualmente, foram detectados vários agentes não previamente descritos, com genomas de DNA fita simples circular, presentemente denominados vírus "CRESS-DNA". Numa etapa seguinte, foi conduzido um estudo visando examinar comparativamente o viroma entérico de frangos de corte afetados pela SMA (n=35) e frangos saudáveis (n=35). Foram detectados genomas de membros das seguintes famílias virais:*Picornaviridae*, vírus CRESS-DNA, *Circoviridae*, *Anelloviridae*, *Reoviridae*, *Picobirnaviridae*, *Astroviridae*, *Caliciviridae*, *Parvoviridae* e *Adenoviridae.* A comparação entre a distribuição do número de *reads* correspondentes as diferentes espécies de vírus eucarióticos identificados no grupo de aves doentes e de aves saudáveis não apresentou diferença estatística. Esses resultados sugerem que a causa da SMA não está relacionada à infecção por um agente viral específico. Futuros estudos são necessários para elucidar a etiologia desta síndrome em frangos de corte.

Palavras-chave: viroma fecal, frangos de corte, distúrbios entéricos, sequenciamento de alto desempenho.

ABSTRACT

Malabsorption syndrome (MAS) is a disease of young broilers characterized by growth retardation, defective feather development and diarrhea with undigested food, resulting in relevant economic impact. Previous studies have investigated the involvement of a number of viral agents in the MAS etiology. However, the limited knowledge of the viral community in poultry gut hinders identification of a particular causative agent(s). In this sense, the present study was conducted aiming to increase the knowledge about the enteric virome of young broiler chickens (3-5 weeks) and its potencial involvement in the occurrence of MAS. For this purpose, on four poultry farms, intestinal contents were collected from clinically healthy chickens. Viral DNA and RNA were extracted, sequenced and compared with previously available data through metagenomic analyzes. Genomes representing a wide diversity of recognized viral families (Adenoviridae, Caliciviridae, Circoviridae, Parvoviridae, Picobirnaviridae, Picornaviridae e Reoviridae) were identified. Also, several agents not previously described were detected, with single-stranded circular DNA genomes, presently known as viruses "CRESS-DNA". In a subsequent step, a study was conducted comparing the enteric virome of broilers affected by MAS (n = 35) and healthy broilers (n = 35). Genomes of members of the following viral families were detected: Picornaviridae, virus CRESS-DNA, Circoviridae, Anelloviridae, Reoviridae, Picobirnaviridae, Astroviridae, Caliciviridae, Parvoviridae e Adenoviridae. Comparison between the distribution of sequences reads matching different species of eukaryotic virus identified in the group of diseased birds and healthy birds did not reach statistical difference. These results suggest the cause of SMA is not related to infection by a specific viral agent. Future studies are needed to elucidate the etiology of this syndrome in broiler chickens.

Keywords: fecal virome, broiler chickens; enteric disorders, high-throughput sequencing.

LISTA DE ABREVIATURAS E SIGLAS

AdVs: adenovírus
AGV2: girovírus aviário tipo 2
ANV: vírus da nefrite aviária
ARV: reovírus aviário
Cap: gene do capsídeo
Cap: proteína do capsídeo
CastV: astrovírus de galinha
CAV: vírus da anemia infecciosa das galinhas
CEUA: Comissão de Ética no Uso de Animais
ChCV: calicivírus de galinha
ChMV: megrivírus de galinha
ChPBV: picobirnavírus de galinha
ChPrV: vírus da proventriculite das galinhas
ChSCV: vírus de genoma circular associado à fezes de galinha
ChSmCV: smaco vírus associado à fezes de galinha
CRESS-DNA: *circular Rep-encoding ssDNA*
CVs: calicivírus
DNA: ácido desoxirribonucleico
FAdVs: adenovírus aviário
GaPV: galliform aveparvovirus
GyV4: girovírus 4
HTS: sequenciamento de alto desempenho
MAS: *malabsorption syndrome*
ME: microscopia eletrônica
NTR: região não traduzida
ORF: fase aberta de leitura
OTU: unidades taxonômicas operacionais
PCR: reação em cadeia da polimerase
RDP: Projeto Banco de Dados Ribossomal
RdRp: gene da RNA polimerase dependente de RNA
Rep: gene da replicase
Rep: proteína da replicase
RNA: ácido ribonucleico
RSS: *Runting stunting syndrome*
RVs: rotavírus
SiV: sicinivírus
SMA: síndrome da má absorção
UTR: região não traduzida
VP: proteína viral

SUMÁRIO

1 INTRODUÇÃO

A indústria avícola consiste no maior e mais avançado acervo tecnológico dentre os diferentes segmentos da pecuária brasileira. Em 2016 o país exportou o equivalente a 4,38 milhões de toneladas de carne de frango, mantendo-se no posto de maior exportador mundial do produto (Abpa, 2017). Os elevados índices produtivos do setor resultam da ação concomitante de nutrição, manejo, genética e sanidade, proporcionando fonte proteica de origem animal de alta qualidade a curtíssimo prazo e a preços acessíveis (Tinôco, 2001).

A tecnificação da cadeia avícola e o rápido desenvolvimento industrial impuseram um aumento exponencial do número de aves criadas por metro quadrado. Esta situação, embora importante para viabilizar os atuais sistemas de produção avícola, favorece a multiplicação e disseminação de agentes patogênicos responsáveis por prejuízos à agroindústria (Andreatti Filho, 2007).

Uma ampla variedade de doenças pode acometer os plantéis avícolas. Entre as enfermidades de maior importância estão os problemas respiratórios e os distúrbios entéricos, responsáveis por implicações econômicas relevantes, como o aumento nas taxas de morbidade e de mortalidade, redução no ganho de peso, aumento da condenação de carcaças no momento do abate, incremento dos custos com medicamentos, mão de obra e serviços técnicos especializados (Alfieri *et al.*, 2000).

Enquanto algumas patologias possuem etiologia bem definida, outras ainda requerem maiores investigações, como a síndrome da má absorção (SMA). Segundo outros autores, esta síndrome tem etiologia multifatorial, caracterizando-se pela presença de múltiplos patógenos atuando de forma sinérgica,sem que até o presente tenha sido possível associá-la à um agente causal único ou primário (Zavala e Sellers, 2005).

A evolução das tecnologias de sequenciamento genético tem permitido a identificação de diversos patógenos previamente desconhecidos. Com estas ferramentas, torna-se possível a detecção de todo o microbioma, ou seja, todo o conjunto de micro-organismos presentes em determinado nicho (Mokili *et al.*, 2012). Nesse sentido, a metagenômica (que compreende o estudo de todos os genomas, genes ou fragmentos destes detectados em determinada amostra) vem sendo utilizada cada vez mais como ferramenta diagnóstica e investigativa, especialmente em síndromes multifatoriais, tais como a SMA (Blomstrom *et al.*, 2010).

O presente trabalho teve como objetivo identificar o viroma (conjunto de vírus), presente no intestino de frangos clinicamente saudáveis, de 3-5 semanas de idade. Além disso, foram examinados comparativamente animais saudáveis (n=35) e animais apresentando sinais de SMA (n=35), com a mesma idade e sob as mesmas condições de manejo. Análises metagenômicas foram realizadas para a caracterização dos genomas detectados.

2 REVISÃO BIBLIOGRÁFICA

2.1 Síndrome da má absorção

O trato gastroentérico apresenta a maior superfície exposta no organismo e está continuamente sujeito à introdução e proliferação de uma infinidade de micro-organismos, sendo a maioria deles benéficos ou comensais. Em determinadas circunstâncias, alguns deles podem causar doenças, enquanto outros, quando presentes, são invariavelmente patogênicos para o hospedeiro (Saif, 2008).

Nas aves de produção, como frangos de corte e perus, o intestino representa um dos principais órgãos sob o ponto de vista econômico. Apesar disso, muito pouco é conhecido sobre a complexa interação da comunidade microbiana que nele habita e o papel desses micro-organismosna indução de doenças (Zavala e Sellers, 2005; Day e Zsak, 2013)

Alterações na saúde intestinal podem acarretar em diminuição da digestibilidade, perdas na conversão alimentar e falha na expressão do potencial genético (Ito *et al.*, 2007; Ito *et al.*, 2009). Consequentemente, há um impacto negativo na produção, o qual pode perpetuar-se por longos períodos mesmo quando ocorre a recuperação clínica das aves (Guy, 1998).

Nas últimas décadas tem ocorrido um aumento progressivo na incidência de doenças entéricas em aves comerciais por todo mundo. Entre essas enfermidades destaca-se a síndrome da má absorção (SMA), inicialmente descritaem frangos de corte no final da década de 1970 (Olsen, 1977; Kouwenhoven *et al.*, 1978; Zavala e Sellers, 2005). Devido à aparente inexistência de um agente etiológico único e à ampla variedade de sinais clínicos observados, a SMA também é conhecida por outros termos, tais como síndrome da desuniformidade tardia (r*unting stunting syndrome* - RSS), síndrome do nanismo infeccioso, síndrome da ave pálida e síndrome dos ossos frágeis (Rebel *et al.*, 2006; Qamar *et al.*, 2013). Este cenário provavelmente está associado a uma série de fatores predisponentes incluindo a racionalização e otimização dos sistemas de produção, tipo de alimento e pressão por seleção genética, fatores que podem favorecer a expressão da patogenicidade de certos agentes (Canelli *et al.*, 2012).

O quadro clínico da SMA é caracterizado por crescimento deficiente, nanismo, desuniformidade do lote e retardo no desenvolvimento das penas devido ao comprometimento da função gastrintestinal (Zavala e Barbosa, 2006). A diversidade de lesões e sinais clínicos varia de acordo com a idade em que o processo é desencadeado.

Frangos de corte nas três primeiras semanas de vida apresentam maior suscetibilidade e tendem a manifestar maiores perdas produtivas. Isso ocorre porque o processo de alongamento das vilosidades intestinais permanece acelerado até a segunda semana de vida. Quando danos ao intestino ocorrem nessa fase, a ave não recupera o desempenho devido ao curto período de vida até o abate (42 dias) (Ito *et al.*, 2009).

Em geral, o dano causado à mucosa resulta em deficiências nutricionais, especialmente aquelas relacionadas às vitaminas lipossolúveis e sais minerais. A carência de vitamina D, por exemplo, impede a absorção intestinal de cálcio o que leva a falhas de mineralização óssea e anormalidades esqueléticas frequentemente vistas em frangos de corte doentes (Ito *et al.*, 2007). Demais sinais clínicos observados incluem a presença de diarreia com resíduos de alimentos não digeridos, palidez de membros inferiores e elevada pigmentação nas fezes quando as aves são alimentadas com milho ou outros alimentos contendo caroteno (Rebel *et al.*, 2006). Retardo do empenamento na região da cabeça, asas e dorso, distensão abdominal, prostração e aumento da mortalidade também podem estar presentes (Frazier e Reece, 1990; Palade *et al.*, 2011). Além dessas alterações, as falhas na digestão e absorção de nutrientes resultam no desenvolvimento inadequado de órgãos linfoides importantes, como a bolsa cloacal e o timo. Como consequência, pode ocorrer imunodepressão, aumento da suscetibilidade a outras doenças infecciosas e elevação na condenação de carcaças ao abate (Guy, 1998).

Durante a necropsia, é possível observar o intestino delgado pálido, distendido, translúcido e com presença de líquidos ou conteúdo não digerido. Ao mesmo tempo, proventriculite, vesícula biliar aumentada e fígado diminuído podem estar presentes (Shapiro *et al.*, 1998).

Alterações histopatológicas são verificadas principalmente no intestino delgado com a presença de lesões císticas nos enterócitos e degeneração das vilosidades intestinais (Figura 1) (Hauck *et al.*, 2016). Outros achados histopatológicos frequentemente encontrados na SMA incluem encurtamento e atrofia das vilosidades, fusão da extremidade dos vilos e distensão das criptas de Lieberkühn (Otto *et al.*, 2006; Sellers *et al.*, 2010). Além disso, são descritas: fibrose, vacuolização e degeneração das células acinares no pâncreas; dilatação celular com infiltração linfocítica no proventrículo e degeneração folicular e epitelial na bolsa cloacal. Entretanto, nenhum desses achados é patognomônico da doença (Qamar *et al.*, 2013).

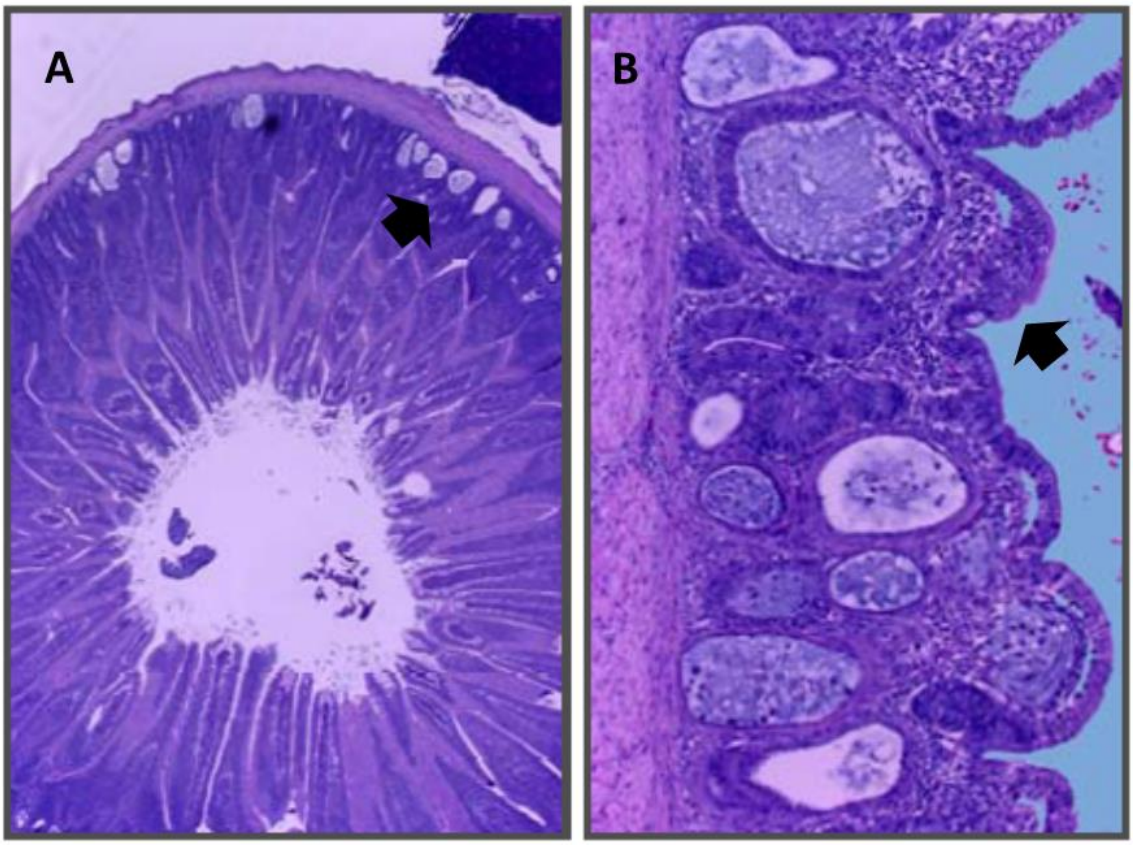

Figura 1 –Alterações histopatológicas verificadas no intestino delgado de frangos de corte afetados pela SMA. Setas indicam presença de lesões císticas na parede intestinal (A) e degeneração das vilosidades intestinais (B) (adaptado de: Zavala, Sellers, 2005).

As causas da SMA e agentes infecciosos potencialmente envolvidos no desencadeamento dessa enfermidade permanecem ainda indefinidos (Rebel *et al.*, 2006; Awandkar *et al.*, 2017). Embora problemas de manejo, falhas nutricionais e fatores ambientais possam estar associados ao desenvolvimento da doença, infecções entéricas causadas por vírus têm sido apontadas como causas primárias desta síndrome (Sellers *et al.*, 2010; Jindal *et al.*, 2014; Devaney *et al.*, 2016). Tal suspeita baseia-se na reprodução do quadro clínico em frangos de um dia de idade após administração de inóculos livres de bactérias, oriundos do conteúdo intestinal de aves afetadas pela SMA (Smart *et al.*, 1988; Reece e Frazier, 1990; Sell *et al.*, 1992; Shapiro e Nir, 1995; Montgomery *et al.*, 1997; Kang *et al.*, 2012).

Na busca de definições sobre a etiologia da SMA, numerosos vírus têm sido isolados ou identificados no conteúdo intestinal de aves afetadas pelo problema, especialmente astrovírus, reovírus, rotavírus e parvovírus (Barnes *et al.*, 2000; Heggen-Peay *et al.*, 2002; Baxendale e Mebatsion, 2004; Cattoli *et al.*, 2005; Nuñez *et al.*, 2016).

Entretanto, tentativas experimentais para reproduzir o quadro com estes agentes, isoladamente, foram inconclusivas (Mettifogo *et al.*, 2014). Em vários estudos, dois ou mais vírus foram detectados simultaneamente indicando que infecções múltiplas podem estar envolvidas na patogênese da doença (Jindal *et al.*, 2009). Por outro lado, a presença desses agentes no intestino de ambos os animais saudáveis e doentes, sugere o envolvimento de diferentes patotipos, ou mesmo a implicação de algum vírus ainda não identificado na ocorrência da SMA (Jindal *et al.*, 2014).

2.2 Agentes virais potencialmente associados à SMA

2.2.1 Astrovírus

Os astrovírus, membros da família *Astroviridae*, são vírus esféricos, icosaédricos e não-envelopados, com aproximadamente 28 a 30 nm de diâmetro (Smyth, 2017). Apresentam um genoma de RNA de fita simples com polaridade positiva, que varia entre 6,8 a 7,9 kb. O termo astrovírus foi cunhado para descrever pequenos vírus arredondados, com projeções com cinco a seis pontas na superfície, semelhantes a estrelas (Méndez e Arias, 2013). No entanto, apenas uma fração dos astrovírus visualizados exibe tal morfologia (Koci e Schultz-Cherry, 2002). A família *Astroviridae* inclui dois gêneros: o gênero *Mamastrovirus*, que engloba os astrovírus de mamíferos, e o gênero *Avastrovirus*, que inclui astrovírus de aves (Bosch *et al.*, 2012). Dentro de cada gênero, numerosos grupos são descritos com base em diferenças sorológicas ou genéticas (Pantin-Jackwood *et al.*, 2011).

O genoma viral possui as extremidades 5’ e 3’ não traduzidas (*untranslated region*, UTR) e três fases de leitura aberta (*open reading frames*, ORFs), variáveis em tamanho, dependendo da cepa (Figura 2). As ORFs 1a e 1b codificam proteínas não estruturais relacionadas com a transcrição e replicação viral, como a enzima serina protease e a RNA polimerase RNA-dependente. A terceira ORF, localizada próximo à região 3’ e designada ORF-2, codifica a proteína do capsídeo (Jindal *et al.*, 2014; Zhang *et al.*, 2017).

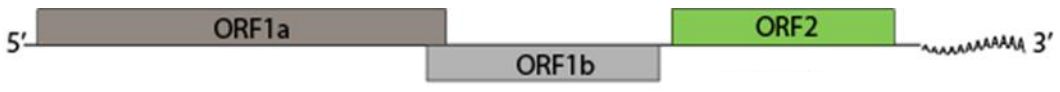

Figura 2 - Organização genômica dos astrovirus (adaptado deViralZone, 2017).

Considerável variabilidade genética é encontrada na ORF-2 dos astrovírus. Essa característica é reportada tanto entre isolados de espécies diferentes quanto entre os sorotipos de uma mesma espécie animal. Com base em predições estruturais, foi sugerido que essa hipervariabilidade na região carboxi-terminal seria responsável pela formação das espículas e, consequentemente, pelas interações precoces entre o vírus e os receptores celulares do hospedeiro (Méndez e Arias, 2013).

Os astrovírus têm sido identificados em uma ampla variedade de espécies animais. Em humanos e mamíferos causam gastroenterites esporádicas, enquanto que em aves, estão associados com doença entérica de maior gravidade, resultando em manifestações extra-intestinais (Oluwayelu e Todd, 2012). Em aves, recentes investigações na etiologia da SMA sugerem o envolvimento de dois astrovírus identificados em galinhas: o vírus da nefrite aviária (*avian nephritis virus*, ANV) e o astrovírus de galinha (*chicken astrovirus*, CAstV) (Pantin-Jackwood *et al.*, 2006; Pantin-Jackwood *et al.*, 2008; Pantin-Jackwood *et al.*, 2011; Canelli *et al.*, 2012; Kaithal *et al.*, 2016).

Em infecções experimentais conduzidas em aves de um dia de idade, os astrovírus induziram redução no ganho de peso, retardo no empenamento e lesões císticas no intestino delgado (Pantin-Jackwood *et al.*, 2006; Sellers *et al.*, 2010). Em outro estudo, três astrovírus (ANV-1, ANV-2 e CAstV) foram detectados por hibridização *in situ* no duodeno de frangos inoculados com conteúdo intestinal oriundo de aves com SMA (Kang *et al.*, 2012). Interessantemente, a administração de uma vacina recombinante expressando a proteína do capsídeo de CAstV protegeu parcialmente a progênie de matrizes submetidas a um modelo de desafio com SMA. Entretanto, a proteção parcial obtida no estudo pode estar relacionada ao fato de existir outros agentes atuando na etiologia da doença (Sellers *et al.*, 2010; Smyth, 2017).

2.2.2 Reovírus

O reovírus aviário (*avian orthoreovirus*, ARV), pertence ao gênero *Orthoreovirus* e à subfamília *Spinareovirinae*, da família *Reoviridae*. Membros dessa família são vírus não envelopados, com simetria icosaédrica e partículas variando entre 70-80 nm de diâmetro (Farkas *et al.*, 2016). O genoma viral dos reovírus possui aproximadamente 23,5 kpb e contém 10 segmentos de RNA fita dupla divididos em três classes: grande (L), média (M) e pequena (S). Os genes L e M são ainda subdivididos em três segmentos cada (L1, L2, L3 e M1, M2, M3), enquanto que o gene S possui quatro segmentos (S1, S2, S3,

S4) (Figura 3). Com exceção de M3 e S1, que originam duas proteínas cada, os demais codificam apenas uma (Benavente e Martinez-Costas, 2007). No total, doze proteínas são traduzidas a partir desses segmentos genômicos, sendo oito delas componentes estruturais dos vírions (λ1, λ2, λ3, μ1, μ2, σ1, σ2 e σ3) e quatro proteínas não estruturais (μNS, μNSC, σ1s e σNS) (Ayalew *et al.*, 2017). A identificação das proteínas codificadas pelos reovírus é designada por letras gregas correspondentes aos tamanhos grande (λ), médio (μ) e pequeno (σ), as quais são traduzidos a partir dos genes L, M e S, respectivamente (Dermody *et al.*, 2013).

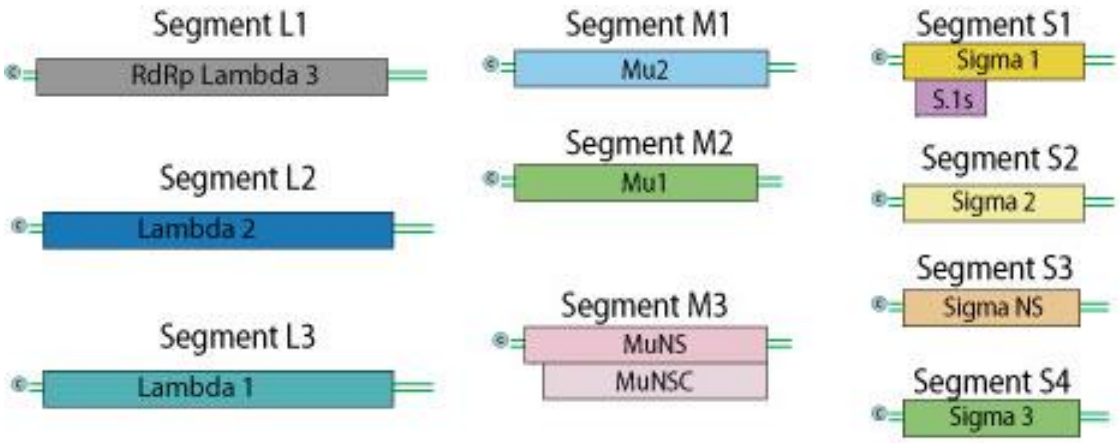

Figura 3 - Representação dos dez segmentos genômicos presentes nos orthoreovírus (adaptado de Viral Zone, 2017).

Reovírus aviários podem ser classificados em dois tipos: fusogênicos e não fusogênicos. O primeiro apresenta a capacidade de causar a fusão das células infectadas resultando na formação de sincícios (células gigantes e multinucleadas), enquanto que o segundo não possui essa habilidade. Reovírus fusogênicos afetam mamíferos, aves e répteis formando grupos geneticamente distintos dos reovírus não fusogênicos, os quais afetam apenas mamíferos(Mor *et al.*, 2014). Em adição, ARVs podem ser diferenciados dos reovírus de mamíferos com base na sua especificidade de hospedeiros e na ausência de atividade hemaglutinante (Alfieri *et al.*, 2007).

Embora ubíquos e frequentemente encontrados em aves saudáveis, os ARVs são implicados em numerosas doenças aviárias, resultando em consideráveis prejuízos para avicultura industrial. Em frangos de corte, a infecção por reovírus frequentemente está associada com artrite/sinovite viral, aumento da mortalidade, diminuição no ganho de

peso, desuniformidade do lote e aumento da taxa de condenação ao abate (Jones, 2008; Day, 2009; Ayalew *et al.*, 2017).

Inicialmente, os ARVs foram considerados os principais agentes etiológicos da SMA em razão de serem isolados ou visualizados por microscopia eletrônica em fezes de galinhas afetadas pela doença (Page *et al.*, 1982; Rekik *et al.*, 1987; Kouwenhoven *et al.*, 1988; Van Loon *et al.*, 2001). Através da inoculação experimental de dois isolados de reovírus (1733 e 2408), foram observados sinais clínicos e lesões intestinais compatíveis com a SMA. Com base nesses achados, atribuiu-se aos ARVs uma função de gatilho no desenvolvimento da síndrome, com infecções bacterianas secundárias ou mesmo outros vírus agravando as lesões intestinais (Montgomery *et al.*, 1997; Songserm *et al.*, 2000; Songserm *et al.*, 2002). Entretanto, a utilização de vacinas contendo esses dois isolados resultou no aumento da performance em apenas 50% dos casos, enquanto que nos casos remanescentes não houve diferença entre aves vacinadas e não vacinadas (Van Der Heide, 2000). Em razão disso, a relação dos ARVs com a ocorrência dessa síndrome permanece pouco esclarecida até o momento (Rebel *et al.*, 2006; Ayalew *et al.*, 2017).

2.2.3 Rotavírus

Rotavírus são membros do gênero *Rotavirus*, pertencentes à subfamília *Sedoreovirinae*, da família *Reoviridae*. Os vírions possuem capsídeo icosaédrico, não envelopado com diâmetro de aproximadamente 85 nm(Attoui *et al.*, 2012). Quando visualizados por microscopia eletrônica, os rotavírus apresentam a aparência semelhante a uma roda, característica que originou o nome do gênero (*rota*, em latim) (Mcnulty e Reynolds, 2008).

O genoma viral possui cerca de 18,5 kpb e consiste em 11 segmentos de RNA fita dupla flanqueados por regiões não traduzidas de extensão variável próximo as extremidades 5’ e 3’ (Figura 4). Cada segmento codifica ao menos uma proteína, totalizando seis proteínas estruturais (*viral protein*, VP) e seis não estruturais (*non-structural protein*, NSP)(Estes e Greenberg, 2013).

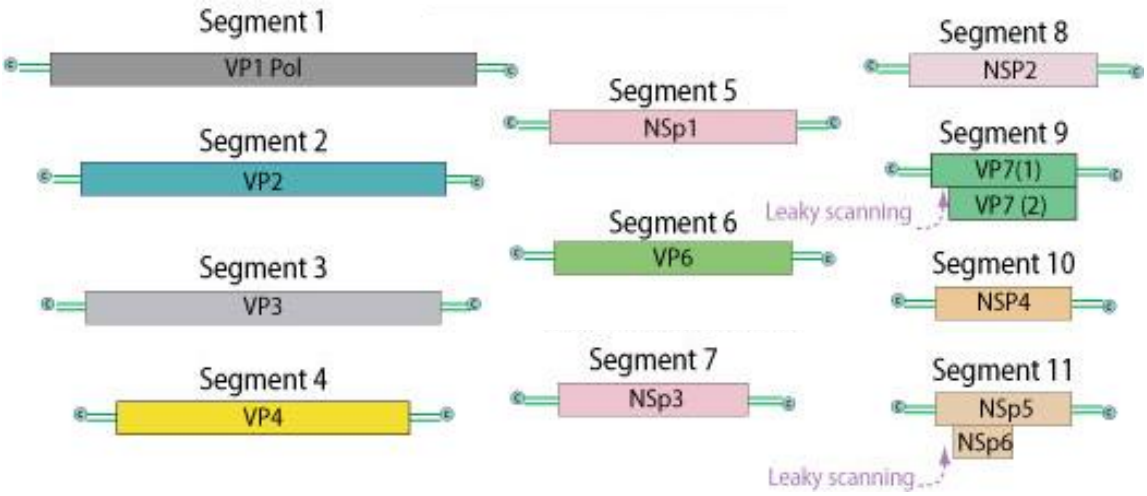

Figura 4 - Representação dos onze segmentos genômicos presentes nos rotavírus (adaptado de Viral Zone, 2017).

A partícula viral é composta por três camadas proteicas. A VP1, juntamente com a VP2 e VP3 encapsidam o RNA genômico formando o núcleo viral, também chamado *core.* A VP6 compõe a camada intermediária e é altamente conservada, o que permite a classificação dos rotavírus em grupos antigênicos distintos. Já a camada externa é constituída pelas proteínas VP4 e VP7, as quais possuem antígenos neutralizantes específicos (Attoui *et al.*, 2012). A VP4 é formada por estruturas diméricas semelhantes a espículas, as quais são responsáveis pela ligação inicial dos vírions aos receptores celulares. Essas estruturas são ativadas pela protease tripsina resultando em aumento da infectividade viral (Johne *et al.*, 2015).

Com base em propriedades antigênicas detectadas na VP6, os rotavírus são divididos em sete grupos sorológicos nomeados pelas letras A aI. (Matthijnssens *et al.*, 2012; Mihalov-Kovacs *et al.*, 2015). Os grupos A, B, C e H são conhecidos por infectar humanos e animais, enquanto que os grupos D, E, F, G e I infectam apenas animais, especialmente aves (Trojnar *et al.*, 2010; Johne *et al.*, 2011; Marthaler *et al.*, 2014; Phan *et al.*, 2017).

Além da classificação em sorogrupos, os rotavírus podem ser divididos em sorotipos ou genótipos, de acordo com características antigênicas e genéticas das proteínas externas do capsídeo. Para isso, é utilizado um sistema binário, similar aquele usado para o vírus da influenza, considerando os tipos de VP4 (ou P - proteína sensível à protease) e VP7 (ou G – glicoproteína) (Matthijnssens *et al.*, 2011). Atualmente são descritos genótipos com mais de 29 tipos de G e 37 tipos de P (Beserra *et al.*, 2014). Esse

sistema de classificação tem permitido verificar a ocorrência de eventos de transmissão entre espécies, bem como recombinação entre os segmentos genômicos de diferentes cepas de rotavírus aviários (Schumann *et al.*, 2009).

Rotavírus foram identificados pela primeira vez apósinoculação experimental de bezerros com inóculo livre de bactérias e obtido a partir de fezes de bezerros apresentando diarreia (Mebus *et al.*, 1969). Desde então, esses vírus têm sido descritos em todo do mundo como principal causa de gastroenterites em crianças e animais jovens (Fischer *et al.*, 2007; Bishop, 2009; Trojnar *et al.*, 2010). Infecções por rotavírus em aves são descritas mundialmente, e assim como em mamíferos, estão associadas a quadros de diarreia com elevadas perdas produtivas no setor avícola (Deol *et al.*, 2017).

Em aves, as alterações clínicas induzidas por esses agentes são bastante variáveis (Guy, 1998). Embora a principal manifestação clínica seja a diarreia, outros sinais podem ser observados: prostração, inapetência, desidratação e emplastamento de cloaca. Como consequência, ocorre diminuição no ganho de peso, desuniformidade dos lotes e aumento da mortalidade (Martins e Resende, 2009).

Rotavírus dos grupos A, D, F e G são frequentemente identificados em diferentes espécies aviárias, como faisões, patos, perus, pombos e aves silvestres (Beserra e Gregori, 2014; Chen *et al.*, 2017; Pauly *et al.*, 2017). Com relação às galinhas, estudos demonstram a disseminação dos rotavírus em lotes comerciais de aves com SMA. Em diferentes lotes acometidos pela síndrome, foi constatada uma correlação positiva entre a presença de rotavírus do grupo D e lesões intestinais graves nas aves examinadas (Otto *et al.*, 2006). Em outra investigação, foram analisadas amostras de 18 perus e 375 frangos afetados pela SMA e oriundos de diferentes países da Europa e de Bangladesh. Dessas amostras, 337 (85%) foram positivas para rotavírus, sendo que destas, 231 (58,8%) positivaram para o grupo A e 259 (65,9%) para o grupo D. Embora existam diferenças geográficas, a alta prevalência de rotavírus A e D constatada nesse estudo indica que esses grupos predominam em aves apresentando sinais clínicos da SMA (Otto *et al.*, 2012).

No entanto, o papel exato dos rotavírus no desenvolvimento da doença permanece pouco esclarecido, uma vez que inoculações experimentais induziram infecções subclínicas (Meulemans *et al.*, 1985; Yason e Schat, 1986). Além disso, a associação destes com a doença é complicada pelo fato de que os rotavírus (assim como outros vírus

entéricos) são frequentemente detectados em aves saudáveis (Panting-Jackwood *et al.*, 2007; Pantin-Jackwood *et al.*, 2008; Day e Zsak, 2013; Mettifogo *et al.*, 2014).

2.2.4 Parvovírus

Parvovírus são vírus pequenos, esféricos não envelopados com cerca de 25 nm de diâmetro e simetria icosaédrica (Berns e Parrish, 2013). O parvovírus de galinha, recentemente nomeado galliform aveparvovirus 1 (GaPV 1), pertence ao gênero *Aveparvovirus* e à subfamília *Parvovirinae*, os quais fazem parte da família*Parvoviridae* (Cotmore *et al.*, 2014).

A estrutura genômica é similar entre os parvovírus da subfamília *Parvovirinae*. Eles apresentam genoma linear composto por molécula de DNA fita simples, 4-6 kb de tamanho e conteúdo de GC variando entre 40 e 55%. As regiões terminais 5'e 3' possuem sequências palindrômicas repetidas (com 120 a 550 nt) as quais dobram-se em estruturas semelhantes a grampos de cabelo (*harpins*), essenciais na replicação e encapsidação do DNA viral (Tijssen *et al.*, 2012).

O genoma dos parvovírus possui dois principais cassetes de genes responsáveis por codificar duas proteínas não estruturais (NS1 e NS2) e três proteínas estruturais (VP1, VP2 e VP3). O GaPV 1 também contém uma pequena ORF de 306 nt, localizada entre as outras duas ORFs maiores, cuja função é desconhecida (Zsak *et al.*, 2009; Day e Zsak, 2010) (Figura 5).

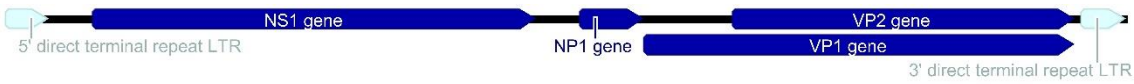

Figura 5 – Organização genômica do parvovírus de galinha (adaptado de Day eZsak, 2010).

A tradução das proteínas NS1 e NS2 ocorre através de *splicing* alternativo dos RNAs mensageiros. NS1 atua na replicação do genoma viral, enquanto que NS2 está envolvida em diferentes funções além da replicação, como por exemplo controle da expressão gênica e formação do capsídeo (Moraes e Costa, 2007). Com relação as proteínas estruturais, VP1 e VP2 são traduzidas a partir de um mesmo RNA mensageiro após mecanismo de *splicing*, enquanto a VP3 é gerada pela clivagem proteolítica da VP2 (Berns e Parrish, 2013). Essas três proteínas compõem a estrutura dos vírions e são

responsáveis pela produção de anticorpos neutralizantes durante a infecção (Saikawa *et al.*, 1993).

Os parvovírus têm sido implicados na etiologia de gastroenterites em uma variedade de mamíferos incluindo cães, gatos e bovinos (Guy, 1998). Infecções por parvovírus também são descritas em perus e galinhas com doença entérica, entretanto, a patogenicidade e importância desses vírus como agente causal requer maiores esclarecimentos.

O potencial envolvimento de parvovírus na SMA foi sugerido após estudo demonstrando a presença de partículas virais semelhantes a parvovírus em fezes de aves jovens afetadas pela doença (Kisary *et al.*, 1984). Posteriormente, frangos de um dia de idade foram infectados experimentalmente com inóculo contendo parvovírus purificado desenvolvendo diarreia, perda de peso (40% menos peso que o grupo controle) e alterações no empenamento (Kisary, 1985). Em estudo recente, resultados semelhantes foram obtidos após a infecção experimental em aves livres de patógenos específicos com uma cepa de parvovírus isolada de galinhas (ABU-P1) (Zsak *et al.*, 2013).

Infecções naturais causadas por parvovírus em lotes de perus e frangos de corte com sinais de enterite foram reportadas na Hungria. Naquele estudo, a investigação histopatológica revelou atrofia de órgãos linfoides e presença de células inflamatórias na lâmina própria do duodeno e jejuno. Além disso, partículas semelhantes a parvovírus foram identificadas por imunohistoquímica indireta demonstrando que o epitélio intestinal dessas aves serviu como local de replicação do vírus (Palade *et al.*, 2011).

Testes em busca de genomas de GaPVstêm revelado que estes agentes estão amplamente disseminados em frangos com sinais clínicos da SMA. A presença de GaPVs foi observada em grande parte das regiões produtoras de aves nos Estados Unidos, sendo as amostras de GaPVs identificadas nesse país, similares geneticamente àquelas circulantes na Hungria (Zsak *et al.*, 2009; Day e Zsak, 2013). A presença de GaPV sem lotes afetados pela SMA também foi descrita em outros países como Croácia e Polônia (Bidin *et al.*, 2011; Tarasiuk *et al.*, 2012). No Brasil, estudos demonstraram que frangos de corte afetados pela síndrome apresentaram maior carga viral do GaPV nas fezes em comparação com frangos saudáveis (Finkler, F. *et al.*, 2016; Finkler, F. *et al.*, 2016).

Entretanto, embora diversas pesquisas tenham sugerido o GaPV como patógeno emergente, seu envolvimento na etiologia da SMA não está claro. Futuros estudos são

necessários para entender a indução de enteropatias ou mesmo infecções assintomáticas causadas por esses agentes (Kang *et al.*, 2012).

2.2.5 Desafios no diagnóstico de agentes virais potencialmente envolvidos na SMA

Em razão de grande parte dos vírus entéricos não serem cultiváveis *in vitro*, os métodos tradicionais de isolamento viral não são utilizados no diagnóstico. Por décadas, a microscopia eletrônica (ME) foi a ferramenta de escolha para identificação destes agentes. No entanto, por não distinguir partículas morfologicamente semelhantes, a ME não permite a identificação de partículas que apresentam variações em sua constituição antigênica (Canelli *et al.*, 2012).

Nesse sentido, os métodos de diagnóstico molecular oferecem vantagens em relação às técnicas convencionais de isolamento, podendo apresentar maior sensibilidade, rapidez e baixo custo (Delwart, 2007). Com relação a SMA, vários ensaios de PCR multiplex têm sido desenvolvidos na tentativa de identificar simultaneamente diferentes patógenos entéricos potencialmente envolvidos no quadro (Day *et al.*, 2007; Panting-Jackwood *et al.*, 2007; Pantin-Jackwood *et al.*, 2008; Jindal *et al.*, 2012). Entretanto, a necessidade do direcionamento prévio da técnica a determinados agentes limita a possibilidade de identificação de novos vírus ou variantes previamente desconhecidas, que possam apresentar potencial patogênico (Blomstrom *et al.*, 2010; Svraka *et al.*, 2010; Day *et al.*, 2015).

Com o advento das técnicas de sequenciamento de alto desempenho e subsequente investigação metagenômica, as limitações associadas aos métodos tradicionais dependentes de isolamento e caracterização viral têm sido contornadas (Rosario e Breitbart, 2011). Essa abordagem possibilita o estudo do conjunto de genomas presentes em determinado nicho ou ambiente, sem conhecimento prévio de sequências (Edwards e Rohwer, 2005). Em razão de permitir a descoberta de vírus “novos”, não cultiváveis, a metagenômica vem se firmando como uma poderosa ferramenta de diagnóstico, podendo ser aplicada tanto para determinação de genomas completos, quanto para investigar a etiologia de doenças complexas (Day *et al.*, 2010; Pallen, 2014; Posada-Cespedes *et al.*, 2017).

2.3 Metagenômica

Micro-organismos não cultiváveis compreendem a maior diversidade biológica da Terra. Até o momento, estima-se que existam em torno de 10^{30} células bacterianas e 10^{31} partículas virais habitando nosso planeta (Riesenfeld *et al.*, 2004; Breitbart e Rohwer, 2005; Carding *et al.*, 2017). Em muitos ambientes, apenas 1% dos micro-organismos podem ser cultivados pelos métodos convencionais, uma vez que as condições laboratoriais tendem a impor pressão seletiva, favorecendo o crescimento de determinados organismos. Com o avanço mais recente de técnicas independentes de cultivo, baseadas em sequenciamento genômico, a composição e diversidade dos microbiomas de diferentes nichos vem sendo desvendados (Singh *et al.*, 2009; Di Bella *et al.*, 2013).

Em 1985, Lane e colaboradores propuseram a investigação do DNA microbiano diretamente de amostras ambientais através de clonagem seguida pelo sequenciamento dos fragmentos encontrados (Lane *et al.*, 1985). Posteriormente, o termo metagenoma foi usado para descrever a coleção de informações genômicas referentes a todos os micro-organismos presentes em uma amostra de solo, incluindo aqueles que não puderam ser isolados (Handelsman *et al.*, 1998). A partir de então, o termo metagenoma tem sido usado para nomear o conjunto de genomas obtidos diretamente de um ambiente ou amostra clínica, enquanto que o termo metagenômica refere-se ao estudo desses dados (Wooley *et al.*, 2010).

Nos últimos anos, a constante evolução e substancial redução no custo das tecnologias de sequenciamento de alto desempenho proporcionaram um aumento no número de estudos envolvendo metagenômica (Thomas *et al.*, 2012; Greninger, 2017). Tal avanço vem sendo verificado em função de que o sequenciamento de alto desempenho *(high-throughput sequencing*, HTS) possibilita sequenciar paralelamente bilhões de moléculas de DNA em um reduzido período de tempo, performance muito superior ao método de sequenciamento convencional (Sanger *et al.*, 1977). Além disso, o HTS dispensa a necessidade de clonagem dos fragmentos de DNA durante o preparo das bibliotecas, reduzindo assim contaminações com ácidos nucléicos oriundos de outros micro-organismos que não fazem parte do metagenoma (Ansorge, 2009; Barzon *et al.*, 2011; Escobar-Zepeda *et al.*, 2015).

Em geral, duas estratégias são utilizadas em estudos metagenômicos: o sequenciamento de genes marcados e o sequenciamento *shotgun*. A primeira refere-se à análise da diversidade microbiana a partir do sequenciamento de *amplicons* alvos, enquanto a segunda corresponde à análise direta dos micro-organismos presentes na amostra. O termo *shotgun* refere-se à quebra aleatória do DNA em pequenos fragmentos durante o preparo das bibliotecas, semelhante ao padrão de disparo de uma espingarda (Dudhagara *et al.*, 2015).

Na metagenômica aplicada ao estudo do viroma, a coleção de vírus presentes em determinado ambiente, são realizadas diferentes etapas de purificação previamente ao sequenciamento. Esse procedimento visa garantir a detecção de ácidos nucléicos virais uma vez que esses encontram-se em pequena quantidade na amostra. O processamento utilizado para concentração de partículas virais inclui as etapas de filtração, ultracentrifugação e remoção enzimática dos ácidos nucléicos desprovidos de capsídeo (Delwart, 2007; Thurber *et al.*, 2009).

Outra técnica utilizada no preparo de amostras inclui a amplificação randômica dos fragmentos após purificação e extração dos ácidos nucléicos. Essa abordagem tem por objetivo acessar os vírus presentes em menor número, bem como, recuperar material genômico suficiente para o sequenciamento (Rosario *et al.*, 2012).

Dessa forma, estudos baseados em metagenômica têm reabastecido o campo da ecologia viral fornecendo um volume de informações sem precedentes sobre a diversidade e as interações entre vírus e hospedeiros (Rosario e Breitbart, 2011). Através dessa técnica, diversos estudos de investigação do viroma foram realizados incluindo amostras ambientais (Breitbart *et al.*, 2004; Alhamlan *et al.*, 2013; Dayaram *et al.*, 2015; Dayaram *et al.*, 2016), alimentos de origem animal (Zhang, W. *et al.*, 2014), trato respiratório (Willner *et al.*, 2009; Yang *et al.*, 2011; Mokili *et al.*, 2013; Correa-Fiz *et al.*, 2016), plasma (Law *et al.*, 2013; Wang *et al.*, 2018), tecidos de animais (Blomstrom *et al.*, 2010; Belak *et al.*, 2013) e fezes de diferentes espécies (Victoria *et al.*, 2009; Shan *et al.*, 2011; Chen *et al.*, 2013; Shah *et al.*, 2014; Zhang, B. *et al.*, 2014; Lima *et al.*, 2017; Moreno *et al.*, 2017). Entretanto, embora a metagenômica represente uma poderosa ferramenta para explorar a diversidade microbiana em diferentes nichos, um dos principais desafios consiste na análise das sequências obtidas (Sharpton, 2014). Os dados produzidos pelas plataformas de HTS correspondem a imensos conjuntos de sequências curtas de leitura (*reads*), o que dificulta a montagem e anotação dos genomas. Em razão

disso, elevados recursos computacionais bem como o uso de *softwares* complexos de bioinformática são requeridos para obter a informação de interesse (Foster *et al.*, 2012).

2.3.1 Bioinformática aplicada àmetagenômica

A análise de bioinformática aplicada aos dados oriundos do HTS segue basicamente quatro etapas: avaliação da qualidade dos *reads*, montagem, classificação taxonômica e anotação funcional. Nesse processo, diversos programas computacionais são utilizados visando obter a identificação precisa de milhares de espécies em um tempo razoável (Cingolani *et al.*, 2015).

A análise de qualidade dos *reads* deve ser realizada antes de qualquer outra análise. Taxas de erros variam entre as tecnologias de sequenciamento e interferem na caracterização da comunidade microbiana. Para estimar a probabilidade de erro de cada nucleotídeo inserido, utiliza-se o escore de Phred. Esse escore classifica os *reads* de acordo com a acurácia na inserção das bases(Bzhalava e Dillner, 2013). Por exemplo, um escore Phred igual a 10 significa que 90% das bases estão corretas, enquanto que os escores 20 e 30 correspondem, respectivamente, a 99% e 99,9% de precisão (Ewing e Green, 1998). Existem vários programas que realizam esse tipo de análise, tais como o FastQC e o PRINTSEQ (Schmieder e Edwards, 2011; Andrews, 2018). Em geral eles fornecem informações adicionais sobre os dados (quantidade e tamanho dos *reads*, conteúdo de GC, sequências super representadas, entre outros) e em alguns casos permitem realizar a trimagem das sequências, ou seja, remoção de adaptadores e/ou regiões de baixa qualidade (Escobar-Zepeda *et al.*, 2015).

Uma vez que os *reads* tenham passado pela análise de qualidade, eles podem ser comparados diretamente com sequências depositadas em bancos de dados públicos ou montadas em sequências contíguas maiores (*contigs*) (Wooley *et al.*, 2010). Diferentes estratégias de montagens estão disponíveis de acordo com o objetivo de cada estudo. Entretanto, dois métodos são frequentemente empregados para montagem de metagenomas: montagem guiada por referência e montagem *de novo* (Miller *et al.*, 2013).

A montagem baseada em referência pode representar uma boa alternativa quando o propósito é recuperar genomas completos ou regiões codificantes de proteínas. Nesses casos, genomas conhecidos podem ser usados para guiar a montagem, desde que o conjunto de dados contenha sequências intimamente relacionadas a eles. Em geral, os algoritmos usados para esse fim são rápidos e possuem memória eficiente o que

possibilita o resultado dentro de poucas horas. No entanto, a presença de grandes variações entre os genomas de referência e os *reads*, devido a ocorrência de inserções ou deleções, resultará em montagens fragmentadas ou em ausência de cobertura nas regiões divergentes (Thomas *et al.*, 2012).

Com relação à montagem *de novo*, maiores recursos computacionais e atenção são requeridos. Isso porque o algoritmo precisa comparar cada sequência com cada leitura diferente, tornando o processo uma operação de alta complexidade (Martins, 2013). Em razão disso, montadores baseados em grafos de Bruijn foram especificamente desenvolvidos para atender o processamento de grandes volumes de dados obtidos no HTS. Esses grafos utilizam um sistema de vértices representados por k-mers (trechos de sequências com tamanho fixo) onde os *reads* atuam como arestas conectando esses vértices. Assim, *reads* sobrepostos são mapeados em uma mesma aresta e podem ser seguidos simultaneamente, resultando na construção de uma sequência consenso do genoma (Wooley *et al.*, 2010). A vantagem em montar os *reads* em *contigs* está relacionada à acurácia da análise. Quanto maior a sequência consenso, maior o ganho de informações facilitando a comparação com dados genômicos conhecidos. Dessa forma, é possível encontrar elementos genéticos complexos e longos ou mesmo reconstruir genomas completos, aumentando a confiança na anotação da sequência (Di Bella *et al.*, 2013).

A investigação da composição taxonômica pode ser realizada através da organização das sequências em classes, ou unidades taxonômicas operacionais (OTU), tornando o método mais rápido e conveniente. Há duas abordagens usadas para esse fim: a clusterização por composição das sequências ou por alinhamento dos *reads* com sequências de referência. A primeira compara todas as sequências entre si e realiza o agrupamento por similaridade, sem utilizar nenhuma referência externa ao conjunto de dados. A segunda, compara as sequências com bancos de dados conhecidos utilizando ferramentas de alinhamento como o BLAST. Dessa forma as sequências são agrupadas em OTU de acordo com a similaridade delas em relação aos dados previamente publicados (Wooley *et al.*, 2010; Thomas *et al.*, 2012).

Outra etapa importante na análise metagenômica, consiste na predição de regiões codificantes, seguida pela anotação gênica. A atribuição de função às ORFs encontradas pode ser realizada tanto em sequências de nucleotídeos quanto em sequências traduzidas. Um dos métodos mais fáceis e utilizados para isso é pesquisa por homologia em bancos

de dados como Swiss-Prot e NCBI-nr, os quais fornecem resultados que também podem ser usados para informação taxonômica (Escobar-Zepeda *et al.*, 2015).

3 OBJETIVOS

3.1 Objetivo geral

Contribuir para a ampliação do conhecimento sobre o viroma fecal de aves comerciais saudáveis e afetadas pela síndrome da má absorção.

3.2 Objetivos específicos

1. Caracterizar o viroma fecal de frangos de corte saudáveis.

2. Caracterizar o viroma fecal de frangos de corte afetados pela síndrome da má absorção.

3. Comparar o viroma de frangos de corte saudáveis e afetados pela síndrome da má absorção, visando examinar o potencial envolvimento de agentes virais na etiologia dessa síndrome

4 CAPÍTULO 1: “Faecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses.”

JOURNAL OF GENERAL VIROLOGY

RESEARCH ARTICLE
Lima *et al.*, *Journal of General Virology*
DOI 10.1099/jgv.0.000711

MICROBIOLOGY SOCIETY

Faecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses

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Abstract

This study is focused on the identification of the faecal virome of healthy chickens raised in high-density, export-driven poultry farms in Brazil. Following high-throughput sequencing, a total of 7743 *de novo*-assembled contigs were constructed and compared with known nucleotide/amino acid sequences from the GenBank database. Analyses with BLASTX revealed that 279 contigs (4 %) were related to sequences of eukaryotic viruses. Viral genome sequences (total or partial) indicative of members of recognized viral families, including *Adenoviridae*, *Caliciviridae*, *Circoviridae*, *Parvoviridae*, *Picobirnaviridae*, *Picornaviridae* and *Reoviridae*, were identified, some of those representing novel genotypes. In addition, a range of circular replication-associated protein encoding DNA viruses were also identified. The characterization of the faecal virome of healthy chickens described here not only provides a description of the viruses encountered in such niche but should also represent a baseline for future studies comparing viral populations in healthy and diseased chicken flocks. Moreover, it may also be relevant for human health, since chickens represent a significant proportion of the animal protein consumed worldwide.

INTRODUCTION

Chickens are major source of animal protein for human consumption worldwide. Feed conversion and poultry productive performance are strongly dependent on the gut microbiota and on adequate functioning of the gastrointestinal tract [1]. In view of this, over the past several years, great efforts have been made to characterize the bacterial population in the chicken gut [2–5]. However, despite the interdependence between bacterial and viral contents in the gut, remarkably little is known about the intestinal virome of poultry [6, 7].

High-throughput sequencing has allowed unprecedented advances in the characterization of complex microbial communities [8, 9]. Such technology, allied to constantly improving methods of metagenomic analysis, has the potential to circumvent the limitations of conventional virological methods, such as the limited ability to replicate most viruses *in vitro*, unsuccessful PCR amplification of viral genomes or failure of antibodies to recognize unknown viruses [10].

Using such an approach, researchers have explored the diversity of the faecal virome from different species at a rapidly increasing rate [10–13]. In turkeys, great strides have

Received 23 November 2016; Accepted 13 January 2017
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Keywords: fecal; virome; broilers; high-throughput sequencing.
Abbreviations: AdV, adenovirus; AGV2, avian gyrovirus 2; CAV, chicken anaemia virus; ChCV, chicken calicivirus; ChPBV, chicken picobirnavirus; CRESS-DNA, circular Rep-encoding ssDNA; FAdV, fowl adenovirus; GyV4, gyrovirus 4; PBV, picobirnavirus; RV, rotavirus; SiV, sicinivirus.
Selected complete or partial genome sequences were submitted to GenBank, and the following accession numbers were obtained: fowl adenovirus RS/BR/2015/1 to 53, KY053058 to KY053110; CAV_RS/BR/15, KY024579; GyV4_RS/BR/15, KY024580; AGV2_RS/BR/2015, KY039279; chicken calicivirus RS/BR/2015, KY120883; chicken smacovirus RS/BR/2015/1, KY086298; chicken smacovirus RS/BR/2015/2, KY086301; chicken smacovirus RS/BR/2015/3, KY086300; chicken smacovirus RS/BR/2015/4, KY086299; chicken stool-associated gemycircularvirus RS/BR/2015, KY056250; chicken stool-associated circular virus_RS/BR/2015, KY056251; galliform aveparvovirus 1RS/BR/2015, KY069111; chicken picobirnavirus strain RS/BR/2015/1, KY123114; chicken picobirnavirus strain RS/BR/2015/2, KY123115; chicken picobirnavirus strain RS/BR/2015/3, KY123116; sicinivirus RS/BR/2015/1, KY069112; sicinivirus RS/BR/2015/2, KY069113; chicken megrivirus RS/BR/2015/1, KY086293; chicken megrivirus RS/BR/2015/2, KY086292; chicken proventriculitis virus RS/BR/2015/1, KY086297; chicken proventriculitis virus RS/BR/2015/2, KY086296; chicken proventriculitis virus RS/BR/2015/3, KY086295; chicken proventriculitis virus RS/BR/2015/4, KY086294; rotavirus A RS/BR/2015/1 to rotavirus A RS/BR/2015/21, KY069090 to KY069110; rotavirus D strain RS/BR/2015/1 to rotavirus D strain RS/BR/2015/22, KY069068 to KY069089.
One supplementary table and three supplementary figures are available with the online Supplementary Material.

been made in the discovery, description and characterization of a multitude of intestinal viruses [14]. As such, a better characterization of the intestinal virome of healthy chickens would be expected to contribute toward increasing productivity by promoting better efficiency of feed conversion, decreasing the possibility of occurrence of disease and, ultimately, increasing food safety by minimizing potential risks to public health [15, 16].

In this study, metagenomic analyses were performed on the faecal virome of healthy, 3- to 5-week-old broilers. The birds were raised in export-driven commercial flocks in Southern Brazil under fairly standard rearing conditions. Sequence data on purified viral nucleic acids extracted from stools were generated in an Illumina MiSeq platform. Similar proportions of viruses of eukaryotes and prokaryotes were identified in samples; however, this report focuses on the description of viral genomes of eukaryotes from chicken faecal samples.

RESULTS AND DISCUSSION

Overview of sequence data

A total of 541 988 paired-end sequence reads with an average of 146.1 nt were generated. Sequence reads were *de novo* assembled and compared against a non-redundant database using BLASTX and BLASTN programmes. Among 7743 assembled contigs, 44.5 % had no similarity to any sequences in GenBank, in agreement with previous findings in human stool samples [10, 17]. Approximately 4 % (279) of the sequences showed similarity to known eukaryotic viral sequences with an E-value cut-off of 10^{-3}. Fig. 1(a) presents the taxonomic classification and the relative amount of the putative viral genomes assembled from the samples examined.

Summary of the findings on the faecal virome of healthy chickens

Fifty-seven percent of the sequences with best BLASTX hits corresponding to eukaryotic viruses were related to DNA viruses of the families *Adenoviridae*, *Circoviridae* and *Parvoviridae*. In addition, a variety of unclassified circular Rep-encoding ssDNA (CRESS-DNA) viral genomes were identified.

Regarding RNA viruses, the identified viral genomes could be assigned to families *Caliciviridae*, *Picobirnaviridae*, *Picornaviridae* and *Reoviridae* (Fig. 1b). Table S1 (available in the online Supplementary Material) presents a summary of the eukaryotic viral sequences detected in the present study.

Next, a brief report on the major groups of viruses identified in the faecal virome of healthy chickens is presented.

DNA genomes

Fowl adenoviruses

Adenoviruses (AdVs) are non-enveloped, icosahedral viruses with a DNA genome consisting of a double-stranded molecule with 26 to 45 kbp [18]. AdVs have been identified in a wide range of vertebrate species, including amphibians, birds, fishes, mammals and reptiles [19]. AdVs that infect chickens are named fowl AdVs (FAdVs). These are currently classified in the genus *Aviadenovirus*, comprising five species (FAdVs A–E) and 12 serotypes (FAdV-1 to 8a and 8b to 11) [20]. In the current study, 53 contigs representing partial genome sequences with high similarity to FAdVs were identified, ranging from 208 to 1217 bp in length (GenBank nos. KY053058 to KY053110).

Of the 53 contigs which could be related to FAdVs, only one corresponded to the genomic region coding for part of the *hexon-associated protein VIII* gene, often used for

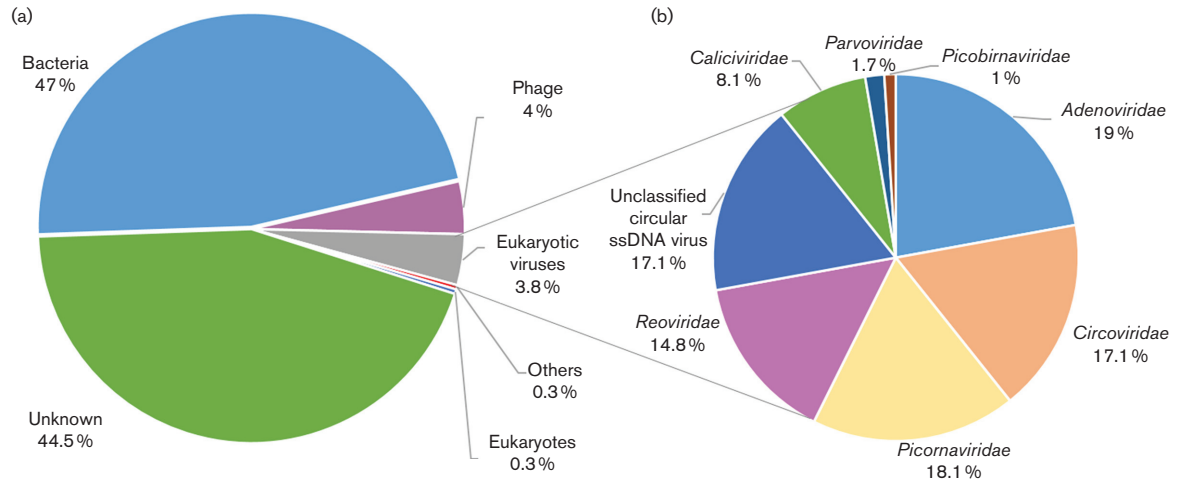

Fig. 1. Putative classification of viral genome sequences detected in pooled stool samples of healthy chickens. Percentages express relative amounts of viral genomes assigned to each particular family (or unclassified) within the total number of contigs obtained. (a) Overview of the *de novo*-assembled Illumina reads. (b) Percentages of eukaryotic viral contigs distributed in different viral families.

phylogenetic analyses [21]. As such, this was the only genomic segment that could be compared with previously reported counterparts. The deduced, partial aa hexon sequence obtained here, named FAdV RS/BR/2015/52, clusters with FAdV-D type 9 (GenBank NC_000899) (Fig. S1).

The presence of FAdVs in faeces of healthy broilers is expected, since FAdV infections are ubiquitous and tend to be asymptomatic in chickens [22]. However, severe disease has been associated to some FAdVs types, especially in young or immunocompromised birds [19, 23, 24], as well as inclusion body hepatitis and hydropericardium in specific pathogen-free chickens [21]. From the findings reported here, it is possible that FAdV RS/BR/2015/52, most likely a type FAdV-D species, may infect healthy birds with no apparent association with disease, since the chickens showed no clinical signs at the time of sampling.

Chicken gyroviruses

Gyroviruses are small non-enveloped DNA viruses with icosahedral symmetry [25]. Such viruses possess circular, single-stranded genomes of ~2.3 kb in length and are classified in the genus *Gyrovirus* within the family *Anelloviridae* [26]. In the current study, the full genomes of gyrovirus 4 (GyV4) and chicken anaemia virus (CAV), as well as a near-complete genome of avian gyrovirus 2 (AGV2), were identified.

The GyV4 full-length genome (named GyV4_RS/BR/15, GenBank KY024580) is 2035 nt long. This genome contains a 518-nt-long non-translated region displaying an average of 55.4 % GC content and a polyadenylation signal (AATAA). Two overlapping ORFs were predicted in the same genome strand (putative VP1, 352 aa long; putative VP2, 217 aa long) (Fig. 2a). A BLASTP search on VP1- and VP2-predicted aa sequences revealed similarities of 99 % and 100 %, respectively, to previously described counterparts in GyV4. Phylogenetic analyses of the *VP1* gene showed that GyV4_RS/BR/15 clustered closely with GyV4 detected in human stool specimens and in chicken meat for human consumption (Fig. 2b) [27]. GyV4 has also been reported in faecal specimens from ferrets, indicating possible dietary sources from consuming infected birds [28, 29]. Therefore, it seems likely that GyV4 may, in fact, be a virus of avian origin, which was incidentally detected in the intestinal contents of other carnivore species, such as reported in the previous studies mentioned above.

The complete CAV genome reported here (strain CAV_RS/BR/15, GenBank KY024579) is 2298 nt in length with a 475-base-long non-translated region and 59.6 % of GC content. The genome organization showed typical features of previously reported CAV genomes, with three overlapping ORFs putatively coding for VP1 (1350 nt), VP2 (651 nt) and VP3 (366 nt) (Fig. 2c). The degree of sequence similarity among CAV_RS/BR/15 and previously reported CAV genomes ranged from 95 % to 100 %. In comparison to the reference CAV genome (accession no. NC_001427), CAV_RS/BR/15 has a 21-nt deletion located within the transcription regulatory region. Such deletion seems associated to a better efficiency to replicate in MDCC-MSB1 cells [30]. However, it is still unclear whether such alteration might affect viral pathogenicity [31, 32]. Phylogenetic analyses based on the VP1 nucleotide sequences cluster CAV_RS/BR/15 into CAV group D, together with isolates from China, USA, Argentina, Japan, Australia and Malaysia (Fig. 2d) [33]. Moreover, the CAV sequence reported here is closely related to the Argentinean isolate 'CAV-10' (accession no. KJ872513), suggesting that both might have a common origin.

The AGV-2-related reads generated a contig (2224 nt) with 98 % to 100 % aa similarity to the previously reported AGV2 sequence (GenBank NC_015396) (Fig. 2e). Phylogenetic trees constructed with basis on the partial VP1 nt sequences indicated that the AGV2 reported here (AGV2 RS/BR/2015, GenBank KY039279) clusters along with AGV2 sequences previously detected in chickens in this same region (Southern Brazil) [34] and in Africa [35] (Fig. 2f). As for GyV4, AGV2 and a number of other gyroviruses have been identified in faeces of humans and ferrets, most likely consequent to the consumption of birds [28, 36]. Although AGV2 has been previously reported in chicken's sera, tissues and feather shafts [34, 37, 38], this is the first report on the identification of AGV2 genomes in the intestinal contents of chickens.

Highly divergent circovirus-like genome sequences

Eukaryotic viruses with circular ssDNA genomes represent 'minimal' viral elements, often with less than 6 kb and encoding a maximum of six proteins [39]. As a result, most ssDNA viruses are highly dependent on the host's replicative machinery and require a conserved replication initiator protein (Rep) for its replication [40]. Viral metagenomic studies revealed a highly diverse population of novel eukaryotic CRESS-DNA viruses in faeces of a number of species, including chimpanzees [41], pigs [12], rodents [42], bats [43, 44], bovines [45], dromedaries [46], humans [47] and caribous [48]. In this study, six full-length genomes of novel CRESS-DNA viruses were identified. Four of these genomes shared 56 % to 94 % aa similarity to the Rep protein of smacoviruses recently reported in faeces from human and non-human primates [49]. Thus, to facilitate description, these four genomes were provisionally named here 'chicken-associated smacoviruses' (ChSmCV RS/BR/2015 from 1 to 4, GenBank KY086298 to KY086301).

One of the other two CRESS-DNA viral genomes identified showed 52 % similarity to the Cap protein of the caribou faeces-associated gemycircularvirus (GenBank NC_024909) and was named 'chicken stool-associated gemycircularvirus RS/BR/2015' (ChSGV RS/BR/2015, GenBank KY056250). The sixth full-length CRESS-DNA identified here was named 'chicken stool-associated circular virus RS/BR/2015' (ChSCV RS/BR/2015, GenBank KY056251) and displayed 42 % similarity to the Rep protein of the rodent stool-associated circular genome virus (GenBank JF755404). All of these six sequences vary in size from 2143 to 4668 nt and contain at least two

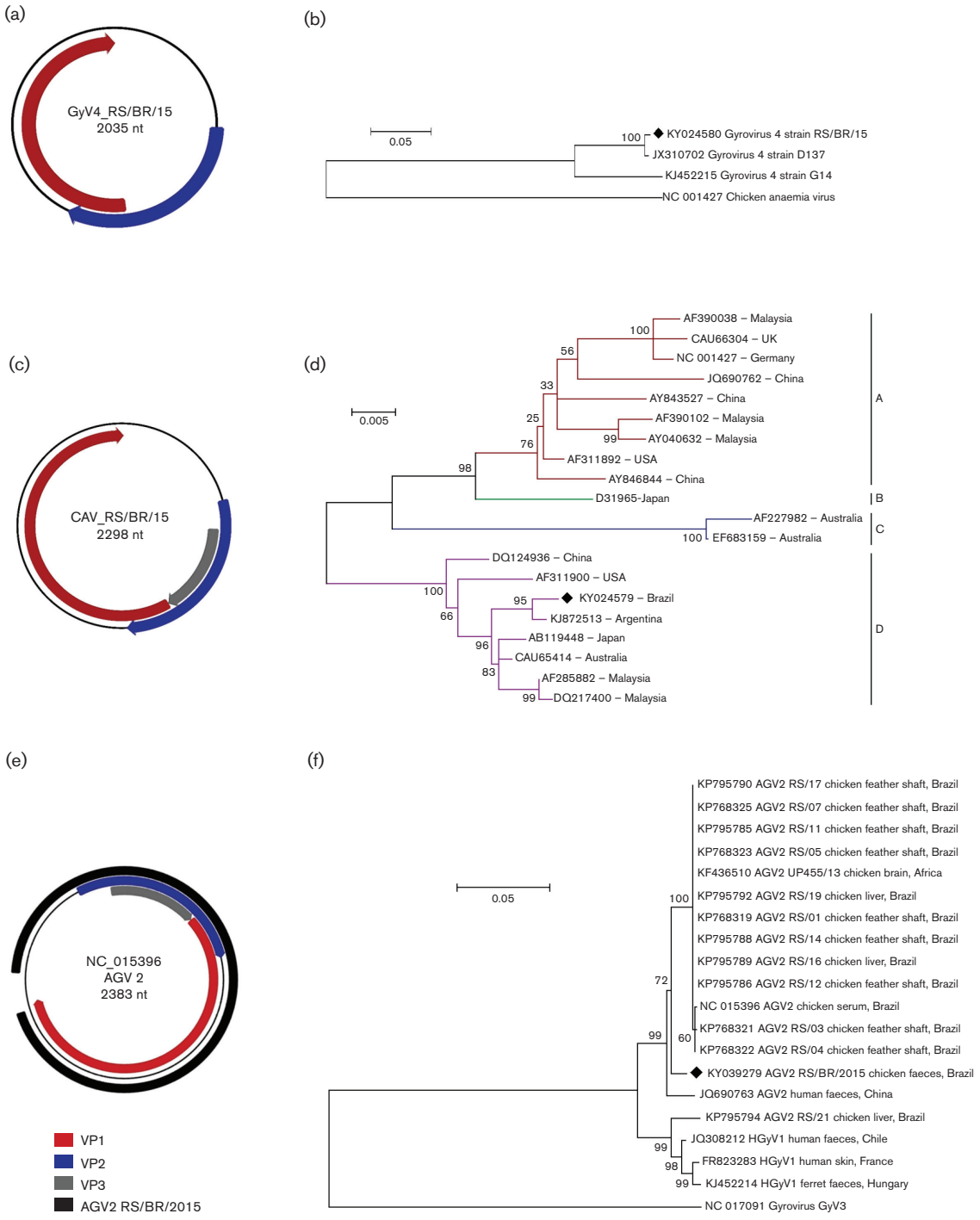

Fig. 2. Putative genome organization and phylogenetic analyses of chicken gyroviruses. (a) Genomic organization of GyV4. (b) Phylogenetic tree based on GyV4 VP1 nucleotide sequences. (c) Genomic organization of CAV. (d) Phylogenetic inferences based on CAV VP1 nucleotide sequences. (e) Schematic representation of the reference genome of AGV2 (NC_015396). The black bar represents the AGV2 genome identified in this study. (f) Phylogenetic tree generated with VP1 nucleotide sequences. Phylogenetic trees were constructed by neighbour joining with a 1000 bootstrap. Sequences identified in this study are highlighted by black diamonds.

ORFs arranged in opposite orientations and encoding two putative proteins: a replicase (204–327 aa) and a capsid protein (305–326 aa) (Fig. 3a). Conserved nonamers at the top of predicted stem-loop structures (Fig. 3b) and two rolling-circle replication motifs II (xHxH) and III (YxxK) were detected in all six genomes.

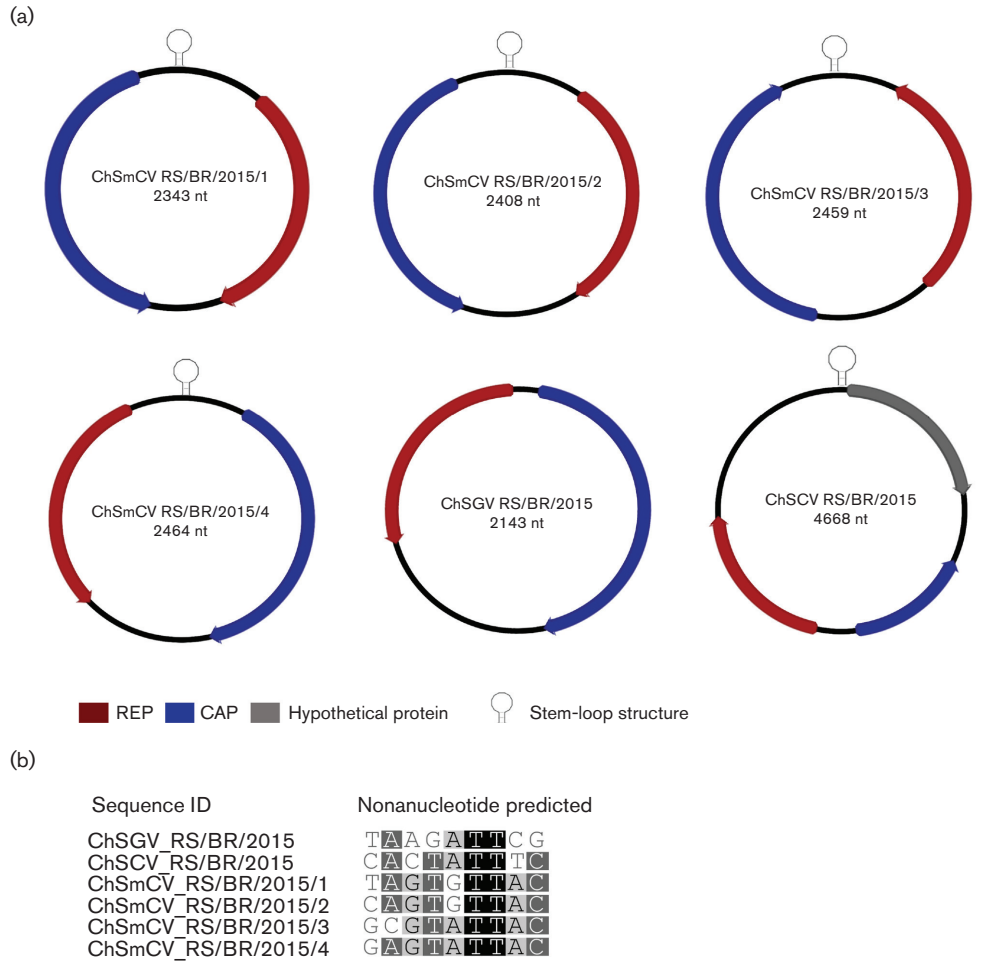

Fig. 3. Novel CRESS-DNA viruses identified in faeces of chickens. (a) Putative genome organization. REP, replication-associated protein gene; CAP, capsid protein gene; hypothetical proteins refer to putative ORFs with no known function. (b) Conserved nonamers identified at the top of predicted stem-loop structures.

The phylogenetic tree constructed based on the Rep-deduced aa sequences allowed clustering of ChSmCV RS/BR/2015/1 along with the turkey stool-associated circular ssDNA viruses [50], while ChSmCV RS/BR/2015/2 to 4 clustered separately, along with smacoviruses detected in human faeces [47] (Fig. 4). Additional phylogenetic analyses showed that ChSCV RS/BR/2015 clusters along with the 'stool-associated circular virus' reported in faeces of dromedaries and rodents [42, 46]. The ChSGV RS/BR/2015 genome clustered more closely to members of the proposed family *Genomoviridae* (previously named *Gemycircularvirus* group) [51]. The first member of the newly described *Genomoviridae* family was isolated from a plant-pathogenic fungus [52] and later reported in sewage, faeces and cerebrospinal fluids from unexplained cases of child encephalitis [48, 53–55].

CRESS-DNA viruses have been detected in the intestinal contents of a broad range of asymptomatic and diarrhoeic animals, including humans [41, 45, 56–59]. Except for the similar sizes and genome organization, little is known about their origin, tropism and biology [50]. Yet, such viruses may be major virome components in most terrestrial and aquatic environments [53]. It is possible that CRESS-DNA viruses in faeces might be related to ingested food or infections of simbiont organisms present in the gastroenteric tract as protozoa, fungi or bacteria [47, 49].

Galliform aveparvovirus

Galliform aveparvovirus 1 (also named chicken parvovirus, ChPV) are small, naked, icosahedral ssDNA viruses, classified in the *Aveparvovirus* genus of the family *Parvoviridae* [60]. Here, a near-complete genome of ChPV comprising the full coding sequence was identified, named ChPV RS/BR/2015 (GenBank KY069111). The assembled contig is 4615 nt long and comprises the full coding region of the ChPV genome (Fig. 5a). The genome architecture of ChPV RS/BR/2015 reveals three ORFs typical of members of the *Aveparvovirus* genus. The first ORF (694 aa,

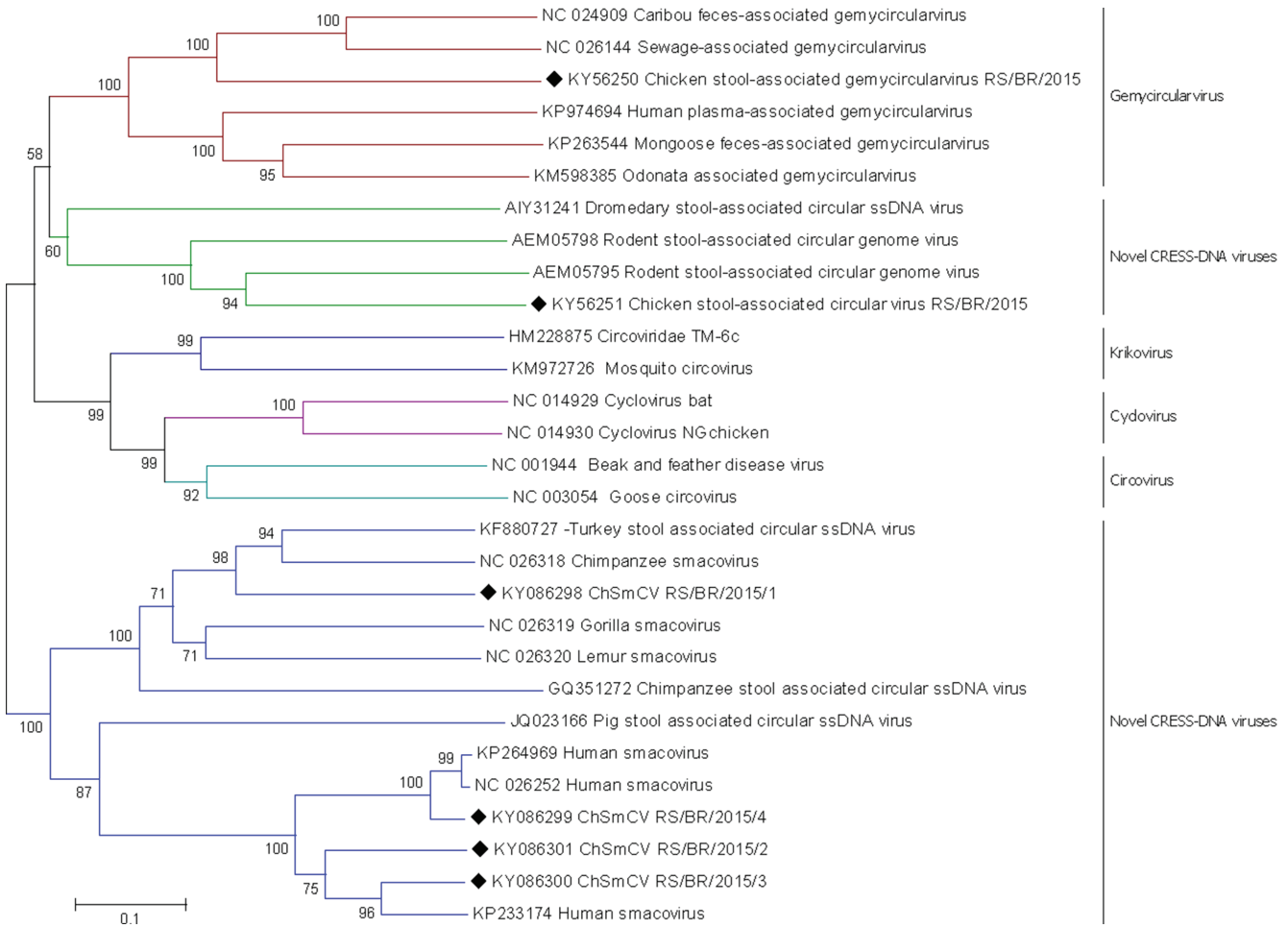

Fig. 4. Phylogenetic analysis of chicken CRESS-DNA viruses identified in faeces of chickens. Phylogenetic inferences based on Rep amino acid sequences were carried out by neighbour joining with a 1000 bootstrap. Sequences identified in this study are highlighted by black diamonds.

78.3 kDa) encodes a putative non-structural (NS) protein with 99 % aa similarity to the prototype sequence ABU-P1 (GenBank NC_024452). The second ORF in ChPV RS/BR/2015 genome shows 87 % aa identity with the NS protein (NP1) of ABU-P1. The third predicted ORF displays 95 % aa similarity to its counterpart in ABU-P1 and encodes two putative capsid proteins VP1 (675 aa, 76.5 kDa) and VP2 (536 aa, 60,4 kDa), believed to be translated from ORF3 by alternative splicing [61].

Phylogenetic analyses based on the nt sequences of the full coding region reveal that ChPV RS/BR/2015 is closely related to galliform aveparvovirus 1 IPV (KU569162), another member of the *Aveparvovirus* genus detected in Southern Brazil. Both formed a distinct cluster from other chicken and turkey parvoviruses previously described (Fig. 5b). Although ChPV has been associated with malabsorption syndrome in broilers, such virus has been detected in both diseased and healthy chickens [62]. In this study, ChPV RS/BR/2015 genome was identified in apparently healthy birds at the time of sampling, which is in agreement with the findings cited above.

To date, few complete ChPV genome sequences are available. The near-complete, full coding sequence of ChPV RS/BR/2015 reported here is an additional source of information for expanding our knowledge on the biology of ChPV.

RNA genomes

Chicken caliciviruses

Caliciviruses are small (27–36 nm), non-enveloped, icosahedral viruses with a linear ssRNA genome [63]. Currently, five genera within the family *Caliciviridae* are recognized by the ICTV: *Nebovirus*, *Norovirus*, *Lagovirus*, *Sapovirus* and *Vesivirus*. In addition, five unclassified caliciviruses (atlantic salmon calicivirus, 'bavovirus', 'nacovirus', 'recovirus' and 'valovirus') have been proposed to form new genera [64, 65]. Here, the complete genome of a new chicken calicivirus (ChCV RS/BR/2015, GenBank KY120883) was identified. Its genome is 8176 nt long (excluding the poly-A tail),

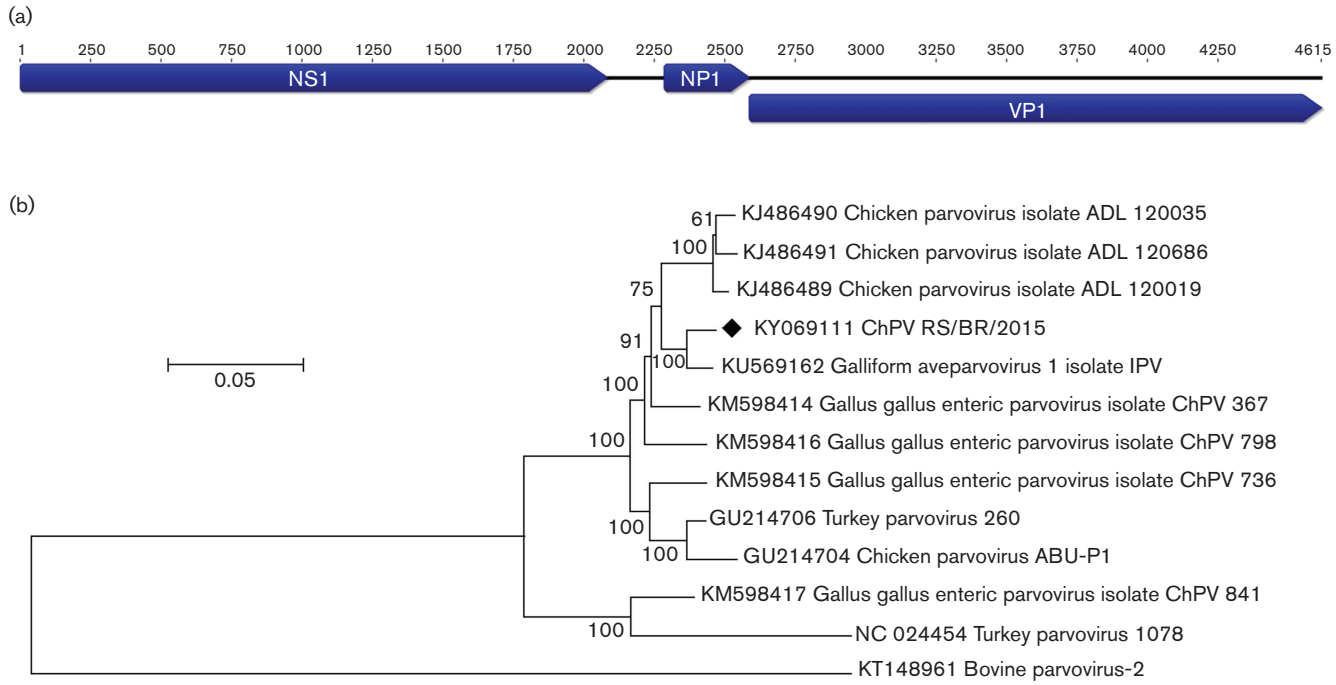

Fig. 5. Genome organization of the putative protein coding region of ChPV RS/BR/2015 and phylogenetic comparison with other parvoviruses. (a) Representation of the complete genomic organization of ChPV RS/BR/2015. The sequence length (4615 nt) corresponds to the full protein coding genome region. (b) Phylogenetic inferences based on nucleotide sequences of the entire coding region were carried out by neighbour joining with a 1000 bootstrap. Black diamond highlights ChPV RS/BR/2015.

with 54 % GC content, in line with mammalian and avian caliciviruses previously reported [66]. The genome architecture of ChCV RS/BR/2015 reveals two main ORFs (Fig. 6a). The predicted ORF1 (2313 aa, 249.5 kDa) encodes a putative polyprotein which is 96 % similar to its counterparts in a previously published ChCV (accession no. KM254171). Some aa motifs typically conserved in CVs' polyproteins were identified, including the NTPase motif GXPGXGKT at position 345, the protease motif GDCGXP at position 1176 and the RNA-dependent RNA polymerase (RdRp) motifs DYSKWDST, GLPSG and YGDD at positions 1457, 1512 and 1560, respectively. The second ORF (287 aa, 30.4 kDa) reveals high similarity (96.1 %) with the *VP2* gene of ChCV strain V0021/Bayern/2004 (GenBank HQ010042). The *VP2* gene encodes a structural protein whose function has not yet been determined [67, 68].

Phylogenetic analyses of ChCV RS/BR/2015 performed using the RdRp aa sequence confirmed that such genomes clustered along with other ChCVs in the proposed *Bavovirus* genus (Fig. 6b). The creation of a new genus within the *Caliciviridae* has been proposed by others to accommodate such viruses [65, 69]. All previously reported ChCV nt sequences deposited at GenBank originated from Germany, Netherlands and Korea. The identification of ChCV RS/BR/2015 in Brazil suggests a widespread distribution of these viruses.

Picobirnaviruses

Picobirnaviruses (PBVs) are small, non-enveloped viruses with bisegmented dsRNA genomes with approximately 4.2 kbp [70]. The large genome segment (S1) encodes the capsid protein, while the small segment (S2) encodes the RdRp. PBVs are highly variable and have been classified in two major groups (GI and GII) based on the S2 genomic sequence [71]. In the present study, three contigs related to PBV genomes were identified (Fig. 2a). One of these, chicken PBV (ChPBV) RS/BR/2015 1 (264 nt, GenBank KY123114), corresponds to a portion of the *capsid* gene and shares 41 % similarity with the equivalent region on the dromedary PBV genome (GenBank KM573789). The other PVB-related sequence, ChPBV RS/BR/2015 2 (258 nt, GenBank KY123115), also matches a portion of the *capsid* gene, displaying 41 % similarity to the homologous region on the porcine PBV genome (GenBank KF861771). The third PBV-related contig, ChPBV RS/BR/2015 3 (GenBank KY123116), shares the highest aa similarity (77 %) with the *RdRp* gene of human PBV (GenBank GQ915028).

Phylogenetic analyses of ChPBV are usually based on the conserved region of the *RdRp* gene [72]. The only PBV-related contig obtained here, which comprised a portion of the *RdRp* gene, named ChPBV RS/BR2015_3, clustered along with members of PBV genogroup II (Fig. 2b).

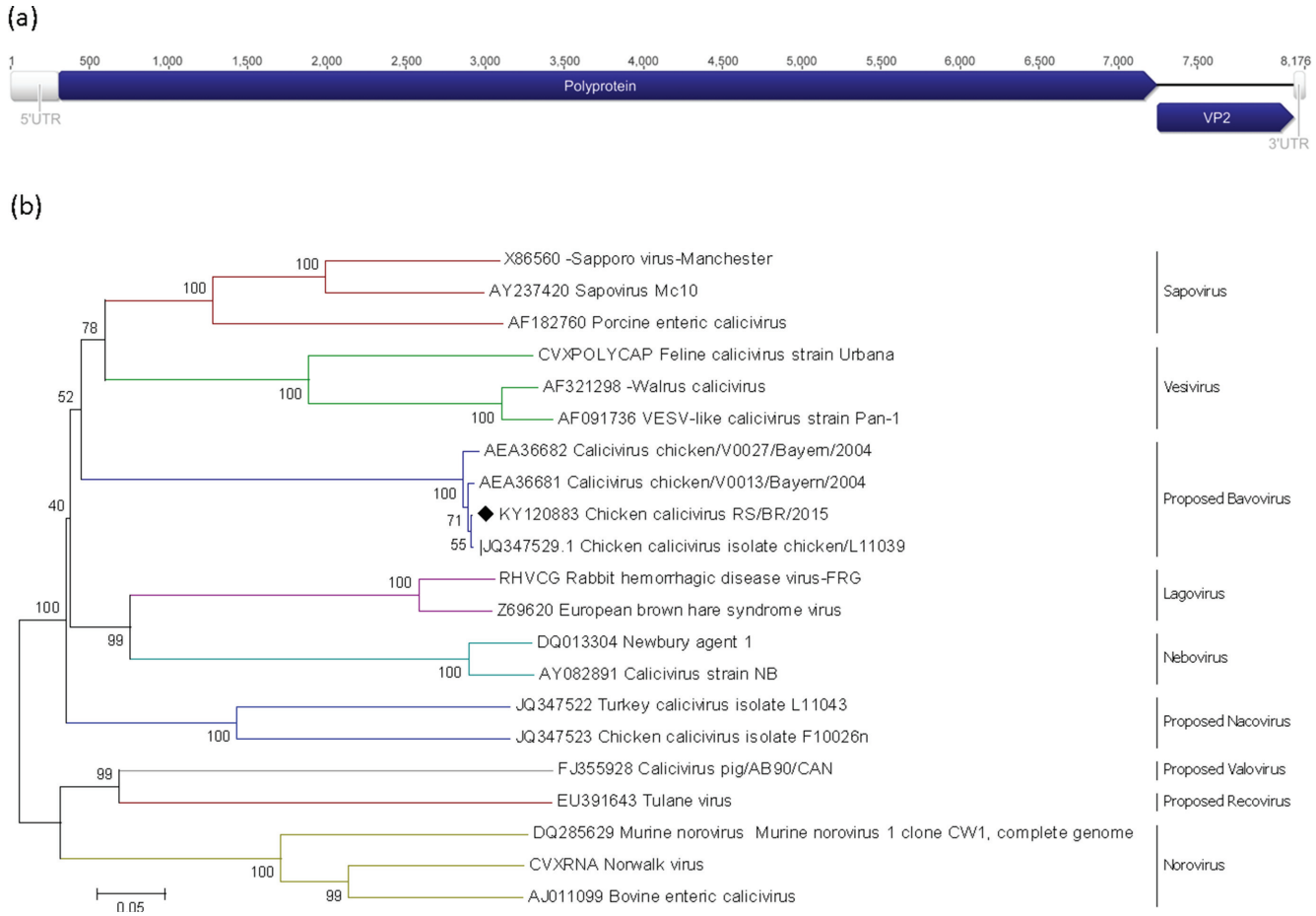

Fig. 6. Putative genome organization and phylogenetic analysis of ChCV strain RS/BR/2015. (a) The genome contains two main predicted ORFs: ORF1 spans almost the complete genome; ORF2 is located at the 3′ end of the genome. (b) Evolutionary relationships based on RdRp aa sequences of ChCV strain RS/BR/2015 with members of the family *Caliciviridae*. Neighbour-joining analyses were performed with 1000 bootstrap replicates. The calicivirus genome introduced here (ChCV RS/BR/2015) is labelled with a black diamond.

PBVs have been detected in faecal samples from both asymptomatic and diarrhoeic or immunocompromised individuals [70]. However, no definite association of PBV with pathogenicity has been drawn [14, 71]. In this study, PBV partial genomic segments were identified in faeces of chickens with no signs of illness, as have other studies [72]. These findings suggest that PBVs are part of the normal intestinal virome of chickens. Further studies should be conducted to investigate the role of PBVs in the intestinal microbiota [46].

Divergent chicken picornavirus genomes

Picornaviruses are non-enveloped viruses with a single-stranded, positive-sense RNA genome. The family *Picornaviridae* currently comprises 31 genera, although new genera are constantly being created [26, 73]. In the last decade, viral genomes of at least eight different genera have been identified in birds: *Avihepatovirus, Avisivirus*, *Gallivirus*, *Megrivirus*, *Oscivirus*, *Passerivirus*, *Sicinivirus* and *Tremovirus*, as well as an unassigned genus comprising phacovirus and chicken picornaviruses 2 and 3 [74]. Nevertheless, little is known about the diversity of picornaviruses in poultry [75]. Here, the near-complete genomes of two novel siciniviruses (SiVs) (genus *Sicinivirus*) and partial sequences which seem related to members of genus *Megrivirus* were identified.

The SiV-related genomes detected in the present study (SiV_RS/BR/2015 1 and 2) reveal a genomic organization common to picornaviruses, with a single large ORF flanked by untranslated regions at both 5′ and 3′ ends, plus a poly (A) tail. The SiV sequence, named RS/BR/2015 1 (GenBank KY069112), is 9253 nt long, with 53.8 % GC content. The predicted large ORF of the genome encodes a putative polyprotein precursor of 2861 aa (314.2 kDa) and shares 85 % aa similarity with SiV A (GenBank NC_023861.1). The second SiV genome identified here, SiV_RS/BR/2015 2 (GenBank KY069113), is 9128 nt in length, with a 53.7 % GC content. A long ORF was predicted, which encodes a 2868 aa (315.2 kDa) putative protein, with 85 % aa similarity to that of SiV A (GenBank NC_023861.1).

The potential protease cleavage sites on the putative polyprotein were mapped by aligning SiV_RS/BR/2015 1 and 2 and other published SiV nucleotide sequences (Fig. 7a). The two SiV genomes reported here share the same layout of predicted cleavage sites and conserved aa motifs as the previously described 2C helicase (GPPGCGKS; DDVGQ), 3C protease (QFKDL; GLCG) and 3D RdRp (KDELR, GGNPSG, YGDD and FLKR) [75, 76].

The $3D^{pol}$ region was used to evaluate the evolutionary relationship between SiV_RS/BR/2015 1 and 2 and other picornaviruses (Fig. 7b). The clustering of SiV_RS/BR/2015 1 and 2 in the phylogenetic tree reveals a close relationship between these two viruses.

In addition to SiVs, other six picornavirus-related sequences were detected, which corresponded to 'chicken megrivirus' (ChMV) and 'chicken proventriculitis virus' (ChPrV), both classified in the genus *Megrivirus* [77]. Two contigs, named ChMV RS/BR/2015 1 and 2 (GenBank KY086293 and KY086292, respectively), showed 96 % to 100 % deduced aa similarity to one of

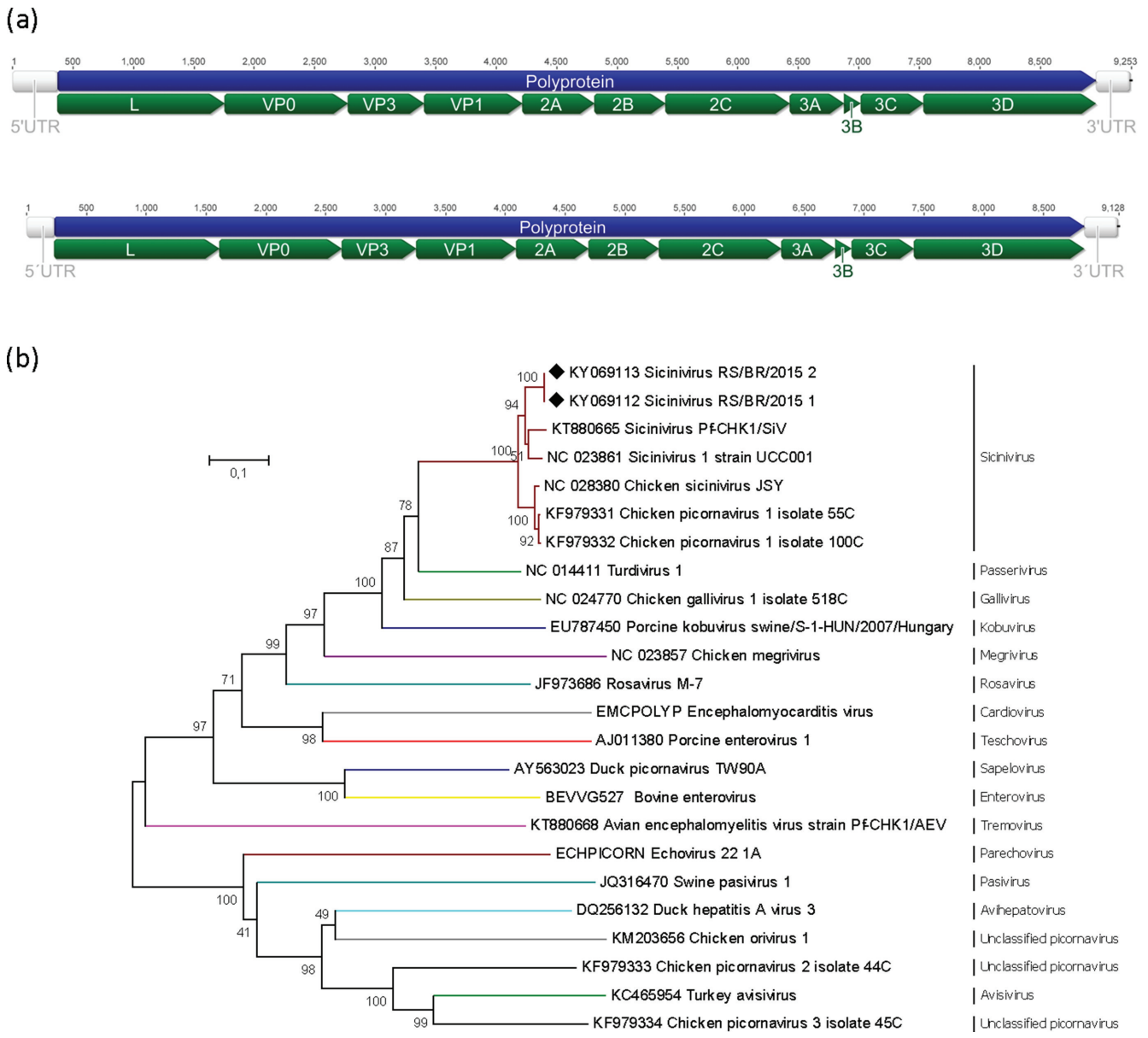

Fig. 7. Putative genome organization and phylogenetic analysis of SiVs RS/BR/2015 1 and 2. (a) Genome organization and predicted cleavage proteins in the polyprotein of SiV RS/BR/2015 1 and 2. (b) Phylogenetic tree generated with basis on the 3D (RdRp-coding) aa sequences, constructed by neighbour joining with 1000 bootstrap replicates. SiV RS/BR/2015 1 and 2 identified in this study are labelled with black diamonds.

the previously published ChMVs (GenBank KF961186). Another four contigs (ChPrV RS/BR/2015 1 to 4, GenBank KY086294–KY086297) shared the highest aa similarity (86–100 %) with ChPrV Korea/03 (GenBank KJ690629) (Fig. 8a). Phylogenetic trees based on the P1 and P2 coding regions, which encode the capsid protein and NS proteins, confirmed that the sequences identified in the current study are closely related to members of the genus *Megrivirus* (Fig. 8b, c).

The wide diversity of picornaviruses in faeces of healthy chickens identified in current study is strongly suggestive of the possibility of recombination events between viruses [74, 78]. However, this remains to be investigated in the future.

Rotaviruses

Rotaviruses (RVs) are non-enveloped viruses classified in the genus *Rotavirus* of the family *Reoviridae* [79]. The RV genome is composed of 11 segments of dsRNA which encodes six structural proteins (VP1–4, VP6 and VP7) and six NS proteins (NSP1–6) [80]. Based on the antigenic characteristics of VP6 protein, RVs have been subdivided into eight serological species (A–H) [81]. In this study, 43 RV-related contigs were identified, ranging in size from 231 to 1589 nt. Among these, 22 shared a high aa similarity (94–100 %) to group D RVs (accession no. KY069068–KY069089), whereas 21 contigs showed 93 % to 100 % identity with group A RVs (accession no. KY069090–KY069110). Phylogenetic analyses based on partial VP6

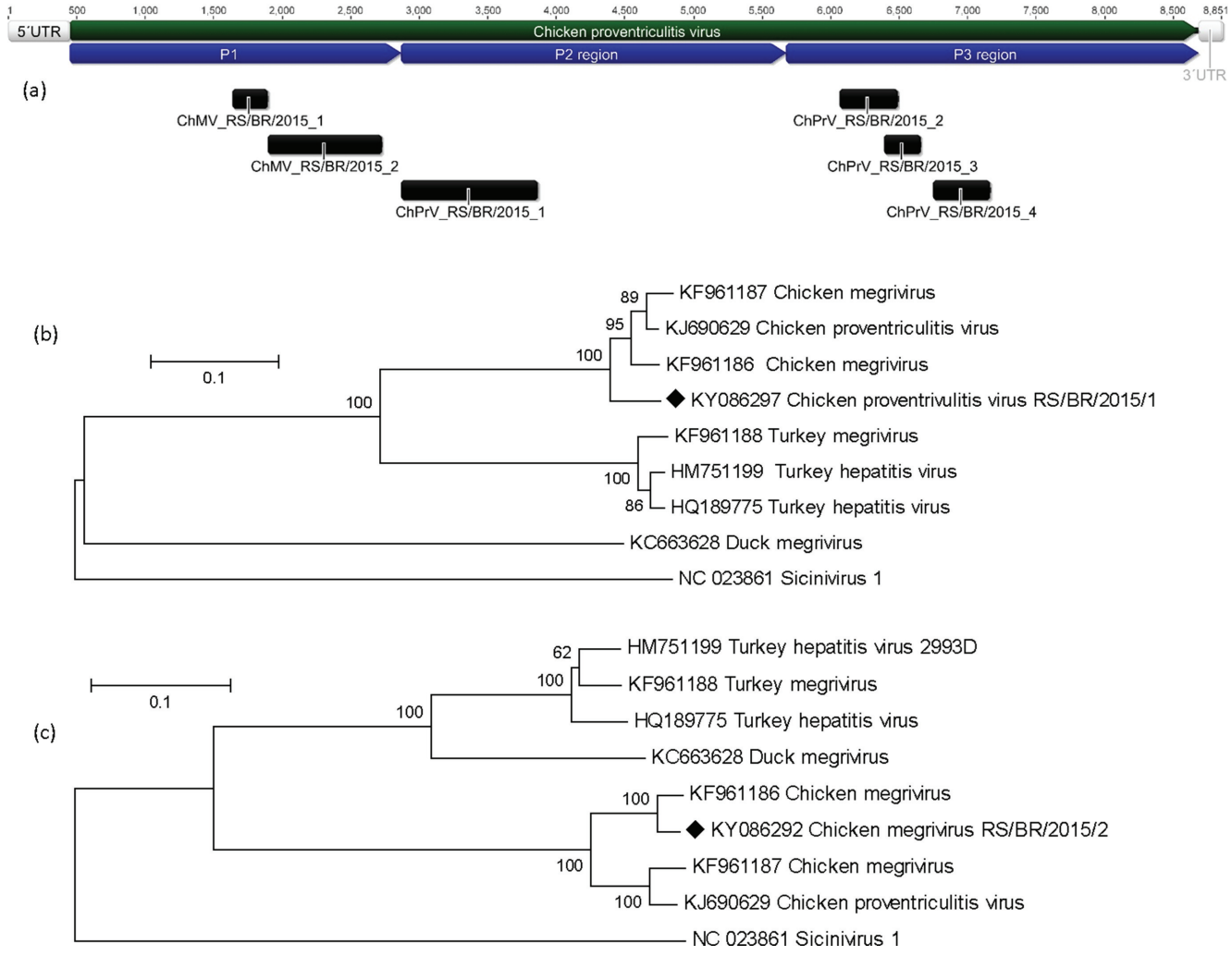

Fig. 8. Phylogenetic analysis of chicken megriviruses. (a) Schematic representation of the genome of megriviruses (ChMV) using chicken proventriculitis virus (ChPrV) as model (~8.8 kb-KJ690629). Black bars represent the contigs identified in the present study. (b) Phylogenetic tree based on the deduced aa sequences of contig ChMV_RS/BR/2015_2 (in a). (c) Phylogenetic tree based on deduced aa sequences of contig ChPrV_RS/BR/2015_1 (in a). Neighbour-joining analyses were performed with 1000 bootstrap replicates. Sequences identified in this study are highlighted with black diamonds.

deduced aa sequences confirmed the putative serogrouping of such viral genomes within groups A and D (Fig. S3).

RVs have been associated to severe diarrhoea in young animals in various species [82]. Group A RVs have often been detected in chickens, humans and other mammals, whereas RV-D are only detected in avian species [83]. Zoonotic transmission, as well as possible recombination/reassortment events, has been demonstrated between human and animal RVs [84, 85]. Infections with distinct groups of RVs, as identified in this study, might represent potential sources for recombination/reassortment events that may contribute to the emergence of additional viral genotypes.

Concluding remarks

The ability to characterize the complexity of the gut microbiome of different species has deeply improved over the past decade, thanks to advances in high-throughput sequencing. By utilizing culture-independent methods, metagenomics has provided relevant information about the composition and diversity of the viral communities in the hosts. In this study, metagenomic analyses of faecal samples of healthy, commercially reared, export-ready chickens revealed a virome constituted by a wide range of viruses representative of known families, as well as a number of novel viruses, most of them with small circular DNA genomes. In this study, the brief description of the genomes of viruses encountered in the faecal virome of healthy chickens presented here is expected to provide a baseline for future studies comparing viral populations of healthy and diseased flocks.

METHODS

Faecal samples

Intestinal contents were collected in four commercial chicken-producing farms in the state of Rio Grande do Sul, Brazil. Birds were produced under standard rearing conditions in commercial flocks, following the protocols of biosafety conditions usually used for export quality flocks. During the year 2015 (May to October), faecal samples were collected from five healthy chickens per house, from birds whose age ranged from 3 to 5 weeks old. Faecal contents were obtained directly from the intestinal tract of euthanized animals and frozen at −80 °C until processing. All procedures were approved by the Commission of Ethics on Animal Use of the Veterinary Research Institute Desidério Finamor (CEUA – IPVDF, no. 21/2014).

Viral nucleic acid purification and Illumina sequencing

Samples were pooled, resuspended in 10 volumes of PBS (pH 7.2) and vigorously vortexed for 5 min. Subsequently, they were centrifuged at 3000 ***g*** for 30 min at 4 °C and filtered through a 0.45 µm filter (Millipore) to remove bacterial cell-sized particles and other particulate debris. The supernatants were centrifuged on a 25 % sucrose cushion at 150 000 ***g*** for 4.5 h at 4 °C (in a Sorvall AH629 rotor), and the pellet containing viral particles was treated with a mixture of 2 µl DNase (2 U μl^{-1}, Turbo DNase; Ambion), 5 µl RNase A (20 mg ml^{-1}; Invitrogen) and 0.5 µl benzonase (25 U μl^{-1}; Novagen) at 37 °C for 2 h to digest susceptible nucleic acids [12]. Viral DNA was then extracted using a standard phenol–chloroform protocol [86]. Viral RNA was extracted with TRIzol LS (Ambion) following manufacturer's instructions.

Viral DNA was enriched by multiple displacement amplification using φ29 DNA polymerase [87]. Viral RNA was converted in cDNA libraries using a SeqPlex RNA amplification kit (Sigma Aldrich) [88]. The DNA products resulting from such enrichment protocols were pooled in equimolar amounts and purified using a Genomic DNA clean and concentrator (Zymo Research). The quality and quantity of the DNA were assessed by spectrophotometry (L-Quant; Loccus Biotechnology) and fluorometry (Qubit; Invitrogen), respectively. The DNA libraries were further prepared with 50 ng of purified MDA/SEqPlex DNA using a Nextera DNA sample preparation kit and sequenced using an Illumina MiSeq instrument (2×150 paired-end reads with the Illumina v2 reagent kit).

Data assembly and processing

The quality of generated sequences was evaluated using FastQC. The sequences with bases having a Phred quality score <20 were trimmed with the aid of Geneious software (version 8.1.7). The paired-end sequence reads were assembled into contigs with SPAdes 3.5 [89]. All assemblies were confirmed by mapping reads to contigs generated by SPAdes using Geneious version 8.1.7 software. The assembled contigs and singlet sequences were examined in search for similarities to known sequences with BLASTX software. Sequences with E-values of $\leq 10^{-3}$ were classified as likely originating from a eukaryotic virus, bacteria, phages, eukaryote or unknown, based on the taxonomic origin of the sequence with the best E-value. ORF predictions and genome annotations of the complete near-full-length genomes were performed with the aid of Geneious software. Gene and protein comparisons were performed with BLASTN and BLASTP programmes.

Reconstruction of phylogenetic trees

Sequences representative of known adeno, calici, circo, CRESS ssDNA, gyro, parvo, picorna, picobirna and RVs were obtained from GenBank and aligned with the sequences identified in the present study with CLUSTALX software version 2.0 [90]. Phylogenetic trees were generated by the neighbour-joining method with a p-distance model. The confidence levels of the tree branch nodes were obtained by analysis of 1000 bootstrap replicates. Analyses were made on MEGA6 [91]. The GenBank accession numbers of the viral sequences used in the phylogenetic analyses are shown on tree figures.

Funding information
This work was supported by the FINEP (project SANIMARS no. 01.10.0783.04 and project MAIS ÁGUA no. 0112.0113), CNPq and

CAPES. D. A. L. has been supported by a CAPES PhD grant. P. M. R. is a CNPq 1A research fellow.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. **Day JM, Oakley BB, Seal BS, Zsak L.** Comparative analysis of the intestinal bacterial and RNA viral communities from sentinel birds placed on selected broiler chicken farms. *PLoS One* 2015;10: e0117210.
2. **Sergeant MJ, Constantinidou C, Cogan TA, Bedford MR, Penn CW *et al.*** Extensive microbial and functional diversity within the chicken cecal microbiome. *PLoS One* 2014;9:e91941.
3. **Oakley BB, Morales CA, Line J, Berrang ME, Meinersmann RJ *et al.*** The poultry-associated microbiome: network analysis and farm-to-fork characterizations. *PLoS One* 2013;8:e57190.
4. **Torok VA, Hughes RJ, Mikkelsen LL, Perez-Maldonado R, Balding K *et al.*** Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. *Appl Environ Microbiol* 2011;77:5868–5878.
5. **Danzeisen JL, Kim HB, Isaacson RE, Tu ZJ, Johnson TJ.** Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS One* 2011;6:e27949.
6. **Shah JD, Baller J, Zhang Y, Silverstein K, Xing Z *et al.*** Comparison of tissue sample processing methods for harvesting the viral metagenome and a snapshot of the RNA viral community in a turkey gut. *J Virol Methods* 2014;209:15–24.
7. **Shah JD, Desai PT, Zhang Y, Scharber SK, Baller J *et al.*** Development of the intestinal RNA virus community of healthy broiler chickens. *PLoS One* 2016;11:e0150094.
8. **Mokili JL, Rohwer F, Dutilh BE.** Metagenomics and future perspectives in virus discovery. *Curr Opin Virol* 2012;2:63–77.
9. **Rosario K, Breitbart M.** Exploring the viral world through metagenomics. *Curr Opin Virol* 2011;1:289–297.
10. **Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B *et al.*** Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. *J Virol* 2009;83:4642–4651.
11. **Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J *et al.*** Metagenomic analyses of an uncultured viral community from human feces. *J Bacteriol* 2003;185:6220–6223.
12. **Shan T, Li L, Simmonds P, Wang C, Moeser A *et al.*** The fecal virome of pigs on a high-density farm. *J Virol* 2011;85:11697–11708.
13. **Zhang B, Tang C, Yue H, Ren Y, Song Z.** Viral metagenomics analysis demonstrates the diversity of viral flora in piglet diarrhoeic faeces in China. *J Gen Virol* 2014;95:1603–1611.
14. **Day JM, Zsak L.** Recent progress in the characterization of avian enteric viruses. *Avian Dis* 2013;57:573–580.
15. **Yeoman CJ, Chia N, Jeraldo P, Sipos M, Goldenfeld ND *et al.*** The microbiome of the chicken gastrointestinal tract. *Anim Health Res Rev* 2012;13:89–99.
16. **Devaney R, Trudgett J, Trudgett A, Meharg C, Smyth V.** A metagenomic comparison of endemic viruses from broiler chickens with runting-stunting syndrome and from normal birds. *Avian pathol* 2016;45:1–14.
17. **Breitbart M, Haynes M, Kelley S, Angly F, Edwards RA *et al.*** Viral diversity and dynamics in an infant gut. *Res Microbiol* 2008;159: 367–373.
18. **Berk AJ.** Adenoviridae. In: Knipe DM and Howley PM (editors). *Fields Virology*, 2 ed. Philadelphia: Lippincott Willians & Wilkins; 2013.
19. **Schrenzel M, Oaks JL, Rotstein D, Maalouf G, Snook E *et al.*** Characterization of a new species of adenovirus in falcons. *J Clin Microbiol* 2005;43:3402–3413.
20. **Hess M.** Detection and differentiation of avian adenoviruses: a review. *Avian Pathol* 2000;29:195–206.
21. **Zhao J, Zhong Q, Zhao Y, Hu YX, Zhang GZ.** Pathogenicity and complete genome characterization of fowl adenoviruses isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China. *PLoS One* 2015;10:e0133073.
22. **Fitzgerald SD.** Adenovirus Infections. In: Fadly AM, Glisson JR, McDouglas LR, Nolan LK, Swayne DE *et al.* (editors). *Diseases of Poultry*. Ames: Blackwell Publishing; 2008. p. 1324.
23. **Domanska-Blicharz K, Tomczyk G, Smietanka K, Kozaczynski W, Minta Z.** Molecular characterization of fowl adenoviruses isolated from chickens with gizzard erosions. *Poult Sci* 2011;90:983–989.
24. **Mettifogo E, Nuñez LFN, Santander Parra SH, Astolfi-Ferreira CS, Ferreira AJP.** Fowl adenovirus group I as a causal agent of inclusion body hepatitis/hydropericardium syndrome (IBH/HPS) outbreak in brazilian broiler flocks. *Pesq Vet Bras* 2014;34:733–737.
25. **Li L, Pesavento PA, Gaynor AM, Duerr RS, Phan TG *et al.*** A *Gyrovirus* infecting a sea bird. *Arch Virol* 2015;160:2105–2109.
26. **ICTV. Virus Taxonomy.** 2015. Release: international committee on taxonomy of viruses; 2016. www.ictvonline.org/virusTaxonomy.asp [accessed 15-nov-2016].
27. **Chu DK, Poon LL, Chiu SS, Chan KH, Ng EM *et al.*** Characterization of a novel gyrovirus in human stool and chicken meat. *J Clin Virol* 2012;55:209–213.
28. **Fehér E, Pazár P, Kovács E, Farkas SL, Lengyel G *et al.*** Molecular detection and characterization of human gyroviruses identified in the ferret fecal virome. *Arch Virol* 2014;159:3401–3406.
29. **Phan TG, da Costa AC, Zhang W, Pothier P, Ambert-Balay K *et al.*** A new *Gyrovirus* in human feces. *Virus Genes* 2015;51:132–135.
30. **Todd D, Connor TJ, Calvert VM, Creelan JL, Meehan BM *et al.*** Molecular cloning of an attenuated chicken anaemia virus isolate following repeated cell culture passage. *Avian Pathol* 1995;24: 171–187.
31. **Zhang X, Xie Q, Ji J, Chang S, Liu J *et al.*** Complete genome sequence analysis of a recent chicken anemia virus isolate and comparison with a chicken anemia virus isolate from human fecal samples in China. *J Virol* 2012;86:10896–10897.
32. **Rimondi A, Pinto S, Olivera V, Dibárbora M, Pérez-Filgueira M *et al.*** Comparative histopathological and immunological study of two field strains of chicken anemia virus. *Vet Res* 2014;45:102.
33. **Eltahir YM, Qian K, Jin W, Wang P, Qin A.** Molecular epidemiology of chicken anemia virus in commercial farms in China. *Virol J* 2011;8:145.
34. **dos Santos HF, Knak MB, de Castro FL, Slongo J, Ritterbusch GA *et al.*** Variants of the recently discovered avian *Gyrovirus* 2 are detected in Southern Brazil and the Netherlands. *Vet Microbiol* 2012;155:230–236.
35. **Abolnik C, Wandrag DB.** Avian *Gyrovirus* 2 and avirulent Newcastle disease virus coinfection in a chicken flock with neurologic symptoms and high mortalities. *Avian Dis* 2014;58:90–94.
36. **Phan TG, Li L, O'Ryan MG, Cortes H, Mamani N *et al.*** A third *Gyrovirus* species in human faeces. *J Gen Virol* 2012;93:1356–1361.
37. **Rijsewijk FA, Dos Santos HF, Teixeira TF, Cibulski SP, Varela AP *et al.*** Discovery of a genome of a distant relative of chicken anemia virus reveals a new member of the genus *Gyrovirus*. *Arch Virol* 2011;156:1097–1100.
38. **Ye J, Tian X, Xie Q, Zhang Y, Sheng Y *et al.*** Avian *Gyrovirus* 2 DNA in fowl from live poultry markets and in healthy humans, China. *Emerg Infect Dis* 2015;21:1486–1488.
39. **Delwart E, Li L.** Rapidly expanding genetic diversity and host range of the *Circoviridae* viral family and other Rep encoding small circular ssDNA genomes. *Virus Res* 2012;164:114–121.
40. **Rosario K, Duffy S, Breitbart M.** A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics. *Arch Virol* 2012;157:1851–1871.

41. Blinkova O, Victoria J, Li Y, Keele BF, Sanz C *et al.* Novel circular DNA viruses in stool samples of wild-living chimpanzees. *J Gen Virol* 2010;91:74–86.
42. Phan TG, Kapusinszky B, Wang C, Rose RK, Lipton HL *et al.* The fecal viral flora of wild rodents. *PLoS Pathog* 2011;7:e1002218.
43. Ge X, Li J, Peng C, Wu L, Yang X *et al.* Genetic diversity of novel circular ssDNA viruses in bats in China. *J Gen Virol* 2011;92:2646–2653.
44. Lima FE, Cibulski SP, Dos Santos HF, Teixeira TF, Varela AP *et al.* Genomic characterization of novel circular ssDNA viruses from insectivorous bats in Southern Brazil. *PLoS One* 2015;10: e0118070.
45. Kim HK, Park SJ, Nguyen VG, Song DS, Moon HJ *et al.* Identification of a novel single-stranded, circular DNA virus from bovine stool. *J Gen Virol* 2012;93:635–639.
46. Woo PC, Lau SK, Teng JL, Tsang AK, Joseph M *et al.* Metagenomic analysis of viromes of dromedary camel fecal samples reveals large number and high diversity of circoviruses and picobirnaviruses. *Virology* 2014;471–473:117–125.
47. Phan TG, da Costa AC, Del Valle Mendoza J, Bucardo-Rivera F, Nordgren J *et al.* The fecal virome of South and Central American children with diarrhea includes small circular DNA viral genomes of unknown origin. *Arch Virol* 2016;161:959–966.
48. Ng TF, Chen LF, Zhou Y, Shapiro B, Stiller M *et al.* Preservation of viral genomes in 700-y-old caribou feces from a subarctic ice patch. *Proc Natl Acad Sci USA* 2014;111:16842–16847.
49. Ng TF, Zhang W, Sachsenröder J, Kondov NO, Da Costa AC *et al.* A diverse group of small circular ssDNA viral genomes in human and non-human primate stools. *Virus Evol* 2015;1:vev017.
50. Reuter G, Boros Á, Delwart E, Pankovics P. Novel circular single-stranded DNA virus from turkey faeces. *Arch Virol* 2014;159: 2161–2164.
51. Krupovic M, Ghabrial SA, Jiang D, Varsani A. *Genomoviridae*: a new family of widespread single-stranded DNA viruses. *Arch Virol* 2016;161:2633–2643.
52. Yu X, Li B, Fu Y, Jiang D, Ghabrial SA *et al.* A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proc Natl Acad Sci USA* 2010;107:8387–8392.
53. Kraberger S, Argüello-Astorga GR, Greenfield LG, Galilee C, Law D *et al.* Characterisation of a diverse range of circular replication-associated protein encoding DNA viruses recovered from a sewage treatment oxidation pond. *Infect Genet Evol* 2015;31:73–86.
54. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U *et al.* Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated sewage. *Virology* 2015;482:98–104.
55. Zhou C, Zhang S, Gong Q, Hao A. A novel gemycircularvirus in an unexplained case of child encephalitis. *Virol J* 2015;12:197.
56. Cheung AK, Ng TF, Lager KM, Bayles DO, Alt DP *et al.* A divergent clade of circular single-stranded DNA viruses from pig feces. *Arch Virol* 2013;158:2157–2162.
57. Cheung AK, Ng TF, Lager KM, Alt DP, Delwart EL *et al.* Identification of a novel single-stranded circular DNA virus in pig feces. *Genome Announc* 2014;2:e00347-14.
58. Li L, Kapoor A, Slikas B, Bamidele OS, Wang C *et al.* Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *J Virol* 2010;84:1674–1682.
59. Sikorski A, Argüello-Astorga GR, Dayaram A, Dobson RC, Varsani A. Discovery of a novel circular single-stranded DNA virus from porcine faeces. *Arch Virol* 2013;158:283–289.
60. Cotmore SF, Agbandje-Mckenna M, Chiorini JA, Mukha DV, Pintel DJ *et al.* The family *Parvoviridae*. *Arch Virol* 2014;159:1239–1247.
61. Koo BS, Lee HR, Jeon EO, Han MS, Min KC *et al.* Genetic characterization of three novel chicken parvovirus strains based on analysis of their coding sequences. *Avian Pathol* 2015;44:28–34.
62. Finkler F, De Lima DA, Cerva C, Cibulski SP, Teixeira TF *et al.* Chicken parvovirus viral loads in cloacal swabs from malabsorption syndrome-affected and healthy broilers. *Trop Anim Health Prod* 2016;48:1685–1689.
63. Tan M, Wei C, Huang P, Fan Q, Quigley C *et al.* Tulane virus recognizes sialic acids as cellular receptors. *Sci Rep* 2015;5:11784.
64. Clarke IN, Estes MK, Green KY, Hansman GS, Knowles NJ *et al.* *Caliciviridae*. In: King AMQ, Adams MJ, Carstens EB and Lefkowitz EJ (editors). *Virus Taxonomy Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses*, 9th ed. San Diego: Academic Press; 2012. p. 1272.
65. Wolf S, Reetz J, Hoffmann K, Gründel A, Schwarz BA *et al.* Discovery and genetic characterization of novel caliciviruses in German and Dutch poultry. *Arch Virol* 2012;157:1499–1507.
66. L'Homme Y, Sansregret R, Plante-Fortier E, Lamontagne AM, Ouardani M *et al.* Genomic characterization of swine caliciviruses representing a new genus of *Caliciviridae*. *Virus Genes* 2009;39:66–75.
67. Glass PJ, White LJ, Ball JM, Leparc-Goffart I, Hardy ME *et al.* Norwalk virus open reading frame 3 encodes a minor structural protein. *J Virol* 2000;74:6581–6591.
68. Sosnovtsev SV, Green KY. Identification and genomic mapping of the ORF3 and VPg proteins in feline calicivirus virions. *Virology* 2000;277:193–203.
69. Wolf S, Reetz J, Otto P. Genetic characterization of a novel calicivirus from a chicken. *Arch Virol* 2011;156:1143–1150.
70. Ganesh B, Masachessi G, Mladenova Z. Animal *Picobirnavirus*. *VirusDisease* 2014;25:223–238.
71. Bányai K, Potgieter C, Gellért Á, Ganesh B, Tempesta M *et al.* Genome sequencing identifies genetic and antigenic divergence of porcine picobirnaviruses. *J Gen Virol* 2014;95:2233–2239.
72. Ribeiro Silva R, Bezerra DA, Kaiano JH, Oliveira DS, Silvestre RV *et al.* Genogroup I avian picobirnavirus detected in Brazilian broiler chickens: a molecular epidemiology study. *J Gen Virol* 2014;95:117–122.
73. Adams MJ, Lefkowitz EJ, King AM, Bamford DH, Breitbart M *et al.* Ratification vote on taxonomic proposals to the international committee on taxonomy of viruses (2015). *Arch Virol* 2015;160:1837–1850.
74. Boros Á, Pankovics P, Adonyi Á, Fenyvesi H, Day JM *et al.* A diarrheic chicken simultaneously co-infected with multiple picornaviruses: complete genome analysis of avian picornaviruses representing up to six genera. *Virology* 2016;489:63–74.
75. Lau SK, Woo PC, Yip CC, Li KS, Fan RY *et al.* Chickens host diverse picornaviruses originated from potential interspecies transmission with recombination. *J Gen Virol* 2014;95:1929–1944.
76. Bullman S, Kearney K, O'Mahony M, Kelly L, Whyte P *et al.* Identification and genetic characterization of a novel picornavirus from chickens. *J Gen Virol* 2014;95:1094–1103.
77. Kim HR, Yoon SJ, Lee HS, Kwon YK. Identification of a picornavirus from chickens with transmissible viral proventriculitis using metagenomic analysis. *Arch Virol* 2015;160:701–709.
78. Boros Á, Pankovics P, Knowles NJ, Nemes C, Delwart E *et al.* Comparative complete genome analysis of chicken and turkey megriviruses (family *Picornaviridae*): long 3′ untranslated regions with a potential second open reading frame and evidence for possible recombination. *J Virol* 2014;88:6434–6443.
79. Otto P, Liebler-Tenorio EM, Elschner M, Reetz J, Löhren U *et al.* Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). *Avian Dis* 2006;50:411–418.
80. Ito H, Sugiyama M, Masubuchi K, Mori Y, Minamoto N. Complete nucleotide sequence of a group A avian rotavirus genome and a comparison with its counterparts of mammalian rotaviruses. *Virus Res* 2001;75:123–138.
81. Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M *et al.* VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. *Arch Virol* 2012;157:1177–1182.

82. **Trojnar E, Otto P, Johne R.** The first complete genome sequence of a chicken group A rotavirus indicates independent evolution of mammalian and avian strains. *Virology* 2009;386: 325–333.
83. **Kindler E, Trojnar E, Heckel G, Otto PH, Johne R.** Analysis of rotavirus species diversity and evolution including the newly determined full-length genome sequences of rotavirus F and G. *Infect Genet Evol* 2013;14:58–67.
84. **Nakagomi O, Nakagomi T.** Genomic relationships among rotaviruses recovered from various animal species as revealed by RNA–RNA hybridization assays. *Res Vet Sci* 2002;73:207–214.
85. **Johne R, Reetz J, Kaufer BB, Trojnar E.** Generation of an avian-mammalian rotavirus reassortant by using a helper virus-dependent reverse genetics system. *J Virol* 2015;90:1439–1443.
86. **Sambrook J, Russel DW.** *Molecular Cloning: A Laboratory Manual*, 3rd ed. USA: Cold Spring Harbor; 2001.
87. **Dean FB, Hosono S, Fang L, Wu X, Faruqi AF *et al.*** Comprehensive human genome amplification using multiple displacement amplification. *Proc Natl Acad Sci USA* 2002;99:5261–5266.
88. **Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T *et al.*** Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. *PLoS One* 2009;4:e4219.
89. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al.*** SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
90. **Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG.** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res* 1997;25:4876–4882.
91. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.

Supplementary Table S1. Putative family assignment of the eukaryotic viral sequence reads detected in fecal samples of healthy broilers.

	Virus family	Total reads
DNA viruses	*Circoviridae*	48,260
	ssDNA circular viruses	797
	Adenoviridae	488
	Parvoviridae	205
RNA viruses	*Picornaviridae*	13,088
	Reoviridae	1,282
	Caliciviridae	1,138
	Picobirnaviridae	12

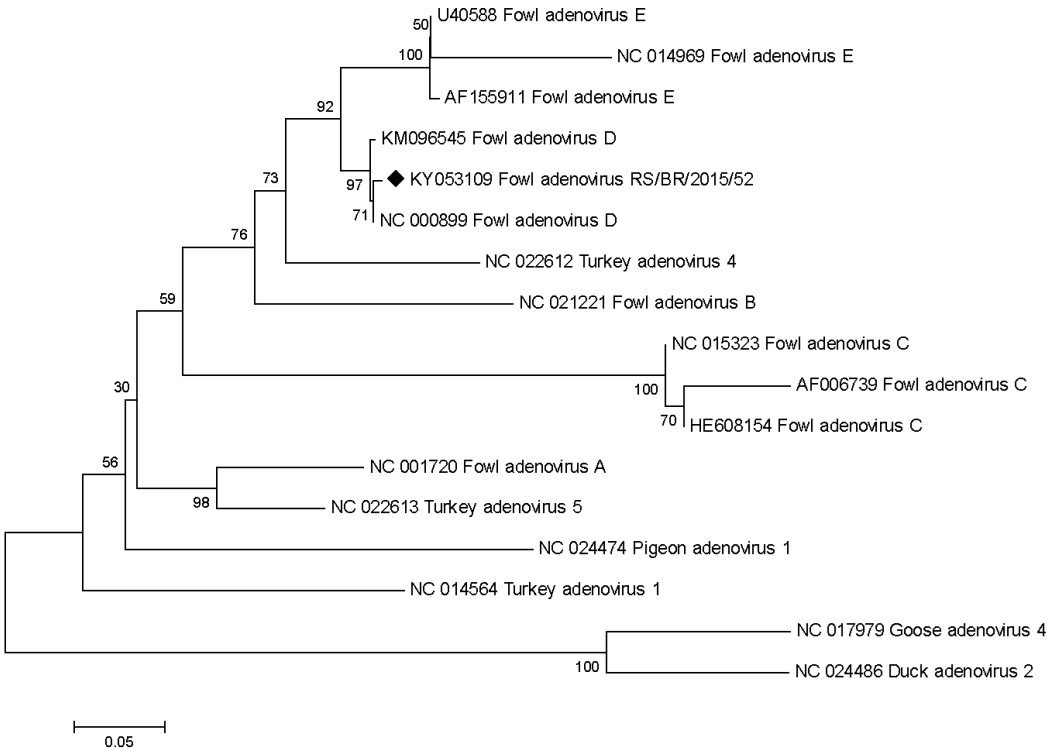

Fig. S1. Phylogenetic analysis of fowl aviadenovirus (FAdV) RS/BR/2015/52. Phylogenetic tree based on the partial amino acid sequences of the hexon-associated protein VIII gene of 17 FAdV. Neighbor-joining analyses were performed with 1000 bootstrap replicates. FAdV RS/BR/2015/52 is highlighted by a black diamond.

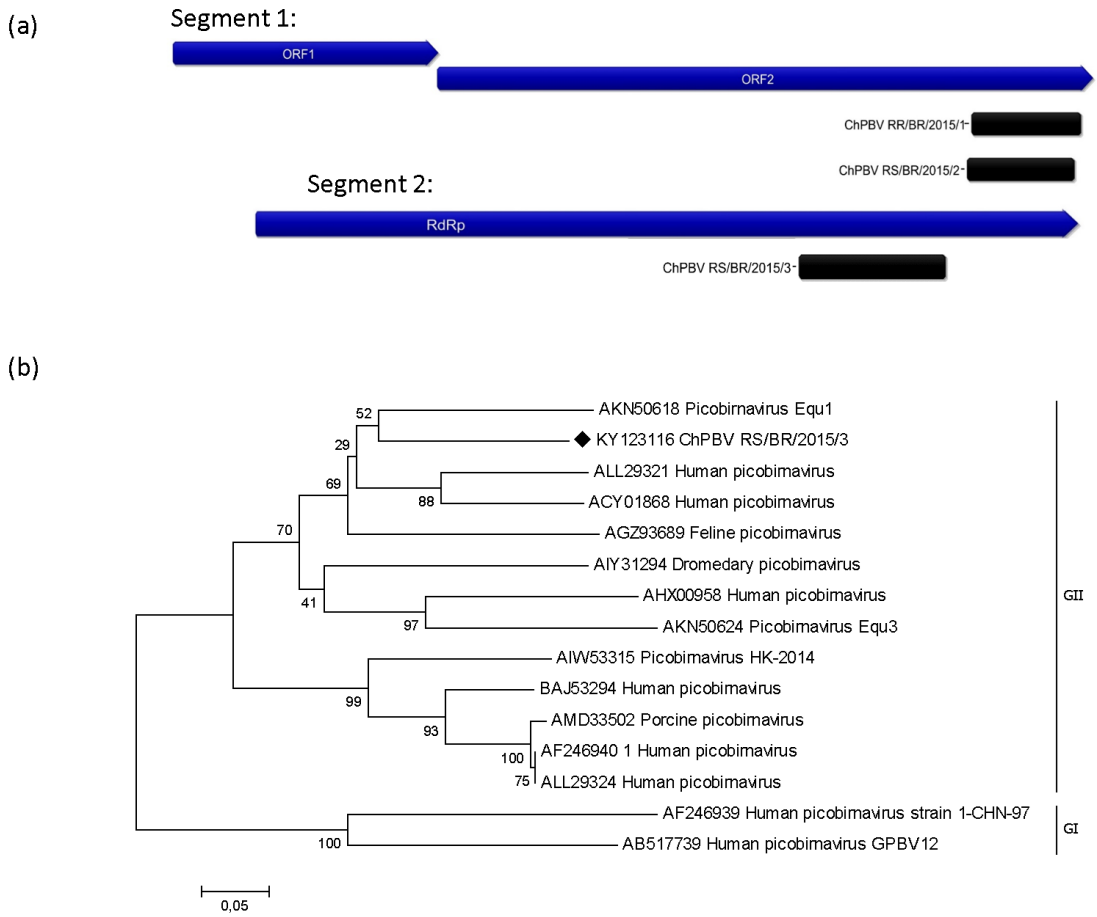

Fig. S2. Phylogenetic analysis of chicken picobirnavirus (ChPBV) RS/BR/2015. (a) Schematic representation of the genome of PBVs using the human PBV (~4.3 kb-NC_007026) as model. The black bars represent the contigs recovered from chicken feces in the present study. (b) Phylogenetic inferences based on the deduced RdRp aa. Inferences carried out by neighbor-joining with a 1000 bootstrap. The ChPBV RS/BR/2015 sequence identified in this study are labeled with black diamonds.

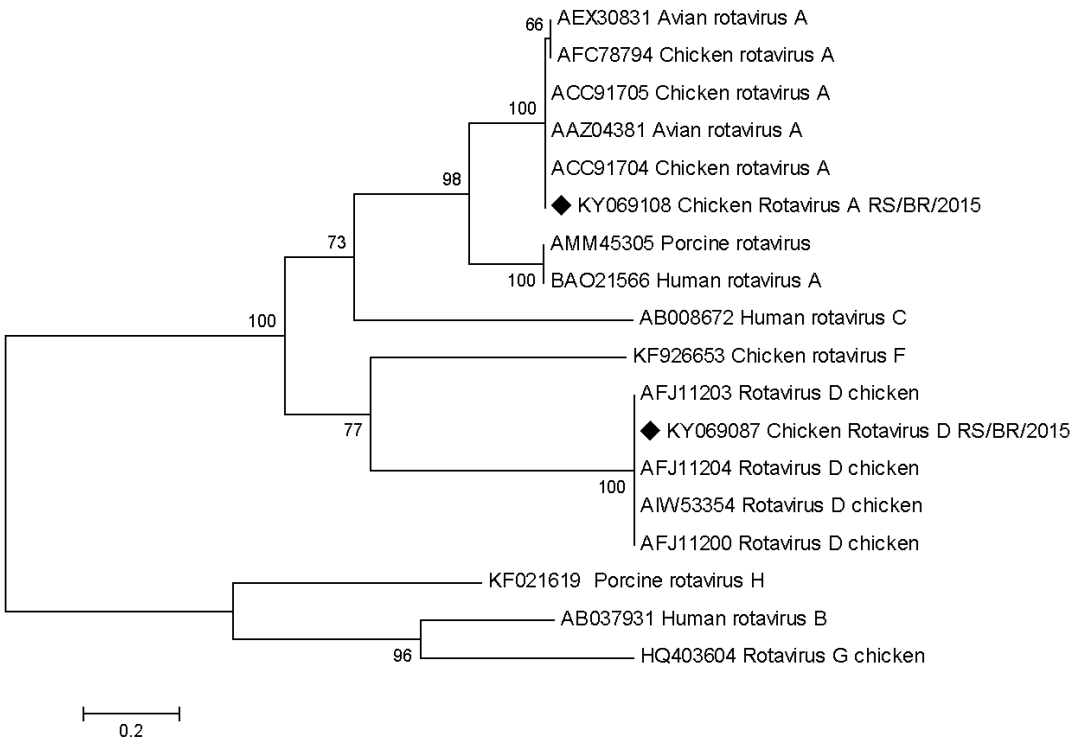

Fig. S3. Phylogenetic analysis of chicken rotavirus (ChRV) A RS/BR/2015 and ChRV D RS/BR/2015. Phylogenetic tree based on the alignment of partial amino acid sequences of the *VP6* gene (segment 6) of 16 rotaviruses. Sequences of groups B, G and H were used as outgroup. The sequences reported in this study are labeled with black diamonds.

5 CAPÍTULO 2: “A case-control study in search for viruses in malabsorption syndrome affected chickens.”

Formatado conforme normas da revista *Journal of General Virology.*

A case-control study in search for viruses in malabsorption syndrome affected chickens

Running title: enteric virome of MAS-affected and healthy chickens

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Word count summary: 239

Word count text: 4,882

No. of figures: 8

No. of supplementary table: 2

No. of supplementary figure: 6

Abreviations: MAS, malabsorption syndrome; CRESS-DNA, circular Rep-encoding ssDNA; ChMGV, chicken megrivirus; ChPhV, chicken phacovirus; ChGV, chicken gallivirus, ChSGmV, chicken stool-associated gemycircularvirus; ChSCV, chicken associated smacovirus; ChCyV, chicken associated cyclovirus; CAV, chicken anemia virus; AGV2, avian gyrovirus 2; GyV4, gyrovirus 4; PBV, picobirnavirus; RdRp, RNA-dependent RNA polymerase; ML, maximum-likelihood; ANV, avian nephritis virus; CAstV, chicken astrovirus; ChCaV, chicken calicivirus; GaPV, galliform aveparvovirus; ChCPV, chicken chapparvovirus; AAAV, avian adeno-associated virus; FAV, fowl aviadenovirus; PCA principal component analyses.

Selected complete or partial genome sequences were submitted to GenBank and accession numbers were obtained:

Chicken megrivirus RS/BR/15/2S to RS/BR/15/3R, MG846455 to MG846465; Chicken sicinivirus RS/BR/15/7R-2 to RS/BR/15/5R, MG846466 to MG846487; Chicken

gallivirus RS/BR/15/2R, MG846488; Chicken phacovirus RS/BR/15/1R-1 and RS/BR/15/1R-2, MG846489 and MG846490; Chicken associated smacovirus RS/BR/15/1R-3 to RS/BR/15/1R-1, MG846351 to MG846354; Chicken stool associated circular virus 2 RS/BR/15/1R, MG846355; Chicken stool associated circular virus 1 RS/BR/15/1R, MG846356; Chicken stool-associated gemycircularvirus RS/BR/15/5S, MG846357; Chicken associated cyclovirus 2 RS/BR/15/4R, MG846358; Chicken associated cyclovirus RS/BR/15/4R to RS/BR/15/5S, MG846359 to MG846362; Chicken anemia virus RS/BR/15/1R, MG846491; Avian gyrovirus 2 RS/BR/15/2S, MG846492; Gyrovirus 4 RS/BR/15/1R and 2S, MG846493 and MG846494; Rotavirus A RS/BR/15/4S-1, MG846363; Rotavirus D RS/BR/15/2R-5 to RS/BR/15/1R-1, MG846364 to MG846378; Rotavirus F RS/BR/15/5R to RS/BR/15/4S-1, MG846379 to MG846387; Avian orthoreovirus RS/BR/15/6S, MG846388; Chicken picobirnavirus RS/BR/15/1S-4 to RS/BR/15/1S-1, MG846389 to MG846412; Avian nephritis virus RS/BR/15/6S-1 to RS/BR/15/6R, MG846413 to MG846415; Chicken astrovirus RS/BR/15/6R-3 to RS/BR/15/6R-1, MG846416 to MG846421; Chicken calicivirus RS/BR/15/5S-2 to RS/BR/15/1R-1, MG846422 to MG846434; Galliform aveparvovirus 1 RS/BR/15/1S to RS/BR/15/1R, MG846435 to MG846440; Chicken chapparvovirus 1 RS/BR/15/6S, MG846441; Chicken chapparvovirus 2 RS/BR/15/2S and 5S, MG846442 and MG846443; Avian adeno-associated virus RS/BR/15/1R, MG846444; Fowl aviadenovirus D RS/BR/15/1R-10 to RS/BR/15/1R-1, MG846445 to MG846454.

Abstract

Malabsorption syndrome (MAS) is an economically important disease of young broilers characterized by growth retardation, defective feather development and diarrheic faeces. Studies have implicated several viruses in the aetiology of MAS; however, the limited knowledge on the microbial community within the poultry digestive trait has hindered identification of a putative causative agent(s) for MAS. In order to examine potential role for viruses in such syndrome, we characterized the faecal virome of 35 stool samples collected from chickens displaying clinical signs of MAS. These were compared to equivalent samples collected from 35 clinically healthy broilers, at the same age and collected in the same flocks (n=7). The overall fractions of eukaryotic viral reads were 22.1% in the MAS-affected group and 14.5% in the healthy group. Genome sequences of a number of previously reported poultry viruses, as well as novel uncharacterized CRESS-DNA viruses, were identified. Genomes representatives of the following families were detected (presented in decreasing order of percentage of reads): *Picornaviridae*, CRESS-DNA viruses, *Circoviridae*, *Anelloviridae*, *Reoviridae*, *Picobirnaviridae*, *Astroviridae*, *Caliciviridae*, *Parvoviridae* and *Adenoviridae*. Comparison between the distribution of sequences reads matching different species of eukaryotic virus identified in the group of diseased birds and healthy birds did not reach statistical difference. These results suggest the cause of MAS is not related to infection by a specific viral agent. Future studies are needed to elucidate the aetiology of this syndrome in broiler chickens.

Keywords: enteric disorders, virome; broiler chickens; high-throughput sequencing.

INTRODUCTION

Malabsorption syndrome (MAS), also known as runting-stunting syndrome, is an economically important disease of young broilers characterized by growth retardation, defective feather development and diarrhea (1). The condition has been reported in chickens worldwide since 1970s (2). Its economic impact is primarily related to inefficient food conversion, decreased meat production, immune dysfunctions and higher mortality rates (3).

For decades, efforts have been made in attempting to identify the aetiology of MAS. However, to date, the causative agent (or agents) remains undetermined (4). Although bacteria, management and environmental factors seem associated to disease development, viruses have been pointed out as major suspects in MAS aetiology (5, 6). Among these, astroviruses (7-9), parvoviruses (10-13), reoviruses (14, 15) and rotaviruses (16, 17) have been in some occasions associated to the occurrence of MAS. However, such viruses have been detected in both healthy and diseased birds, making it difficult to establish direct causal relationships (18, 19).

More recently, viral metagenomics has been successfully applied to characterize viral populations in different hosts and environments (20, 21). Such studies have significantly contributed to increase knowledge on a great diversity of viruses, allowing for the discovery of impressive numbers of previously unknown agents in various tissues and organs, including the gastrointestinal tract of broilers and other animals (5, 22-30). Metagenomics has also been used to investigate the aetiology of diseases with no association to previously known causative agents had been reported (31-34). In the present study, a metagenomic approach was employed to characterize the intestinal virome of chickens with and without MAS in a case control study. Faecal samples were collected from diseased and healthy birds and examined comparatively.

RESULTS

Viral metagenomics

Faecal samples from MAS-affected chickens (n=35) and healthy controls (n=35) were used to prepare the viral metagenomic libraries. Samples were pooled according to the farm of origin and clinical condition (named G1 to G7 "R" or "S"), resulting in 14 pools. A total of 8,347,319 paired end reads, with an average of 231 nt, were generated using the Miseq sequencing platform. Through analysis of *de novo* assembled contigs, 144 contigs > 1,000 nt were identified with hits to eukaryotic viral sequences according to BLASTx analysis. The percentage of eukaryotic virus reads detected in each group was 22,1% in the MAS-affected against 14,5% in the healthy group. Sequences related to nine known viral families and CRESS-DNA viruses were identified; their relative frequencies in each of the groups (MAS-affected and healthy birds) are shown in Fig. 1. The following viral families/groups were detected (in decreasing percentage of abundance of reads): *Picornaviridae*, CRESS-DNA viruses, *Circoviridae*, *Anelloviridae*, *Reoviridae*, *Picobirnaviridae*, *Astroviridae*, *Caliciviridae*, *Parvoviridae* and *Adenoviridae*. The greatest number of viral hits in MAS-affected group were from members of the *Picornaviridae*, comprising 74% of all eukaryotic virus sequences detected, against 35% in healthy birds. Another difference was the percentage of reads of CRESS-DNA viruses: 15% in MAS-affected birds, against 39% in healthy ones. Moreover, representatives of the *Circoviridae* were detected in 3% of the reads in MAS-affected birds, whereas in 10% of the sequences from healthy birds.

Complete and partial viral genomes

(i) *Picornaviridae:*

Megrivirus: A total of eleven contigs ranging in size from 1,226 to 9,577 nt were identified based on protein similarity (Table S2). Complete or nearly complete genomes were generated in four contigs, named ChMGV/RS/BR/15 2R, 3R, 5R and 3S/2 (Fig. 2a). The ChMGV/RS/BR/15-2R, 3R and 3S/2 showed 96%, 93% and 98% identity at amino acid (aa) level to the polyprotein of chicken proventriculitis virus (Genbank ac. no.KJ690629), respectively. The ChMGV/RS/BR/15 5R displayed 96% aa identity to chicken megrivirus polyprotein (Genbank ac. no. KF961186).

Sicinivirus: Seven complete (9,075 nt to 9,874 nt) and one partial (8,710 nt) coding sequence (CDS) of sicinivirus were detected (Fig. 2a). Fourteen shorter partial genomes were also identified (Table S2). These viruses shared between 77% and 98% aa identity to polyprotein of other siciniviruses available at GenBank (Table S2).

Phacovirus: Two phacovirus-related contigs were detected. One of these sequences (ChPhV/RS/BR/15/1R-1, 4,104-nt-long) contained 5'UTR and encode the first half of the phacovirus genome (Table S2). The second contig (ChPhV/RS/BR/15/1R-2, 3,925-nt-long) has 3'UTR and represent the final portion of phacovirus genome (Fig. 2a). At the aa level, ChPhV/RS/BR/15/1R-1 displayed 87% identity to polyprotein of chicken phacovirus (GenBank ac. no. KT880670), while ChPhV/RS/BR/15/1R-2 shared 99% aa identity to the same genome.

Gallivirus: A 2,226 nt-long contig (ChGV/RS/BR/15/2R) of chicken gallivirus was detected (Fig. 2a). The sequence shows a 3'UTR and encoding a partial polyprotein which shares 99% aa identity to the prototype strain of chicken gallivirus 1 (GenBank ac. no. NC_024770).

The sequences described here and picornaviruses from different genera were used for phylogenetic analysis performed using 3D polymerase amino acid sequences. Phylogenetic tree is shown supporting their genera assignments (Fig. 2b).

(ii) Unclassified CRESS-DNA viruses: Seven complete circular genomes of CRESS-DNA viruses were detected. All of these genomes encoding a Rep and a Capsid protein bidirectionally organized and varying in size from 2,161 nt to 3,173 nt. The genome characteristics are shown in Fig. 3(a). Table S2 presents the aa similarity with their matches deposited in GenBank.

A maximum-likelihood phylogenetic tree was reconstructed based on alignments of the putative Rep amino acid sequences (Fig. 4). One Rep sequence (chicken stool-associated gemycircularvirus, ChSGmV, RS/BR/15/5S) clustered with members of the recently described *Genomoviridae* family (35), while other four sequences (chicken associated smacovirus, ChSmV, RS/BR/15/1R-1 to 4) were more closely to smacoviruses (36). The two remaining sequences (chicken stool associated circular virus, ChSCV, RS/BR/15/1R-1 and 2) were more closely related a distinct cluster of CRESS-DNA viruses reported in faecal samples from Peruvian patients with diarrhea (37).

(iii) *Circoviridae*: Five full genomes of cycloviruses (1,778 nt to 1,905 nt-long) were recovered in this study (Fig. 3b). The major ORFs (putative Rep and Cap proteins) are arranged in opposite directions and are separated by an intergenic region that contain the putative origin of replication. Four sequences (named chicken associated cyclovirus, ChCyV_RS/BR/15/4R, 2S, 4S and 5S) shared 98-99% aa identity to the Rep protein of Duck associated cyclovirus 1 (GenBank no. ac. NC_034977). The other cyclovirus contig showed only 48% aa identity to the putative Rep of dragonfly associated cyclovirus 6 (GenBank no. ac. KC512918) and was provisionally named chicken associated cyclovirus 2 (ChCyV 2 RS/BR/15/4R).

The phylogenetic analysis of the cyclovirus Rep protein described above is shown in Fig 4. Maximum-likelihood phylogenetic tree confirmed that sequences ChCyV described here are closely related to duck associated cyclovirus 1, which form a monophyletic cluster. On the other hand, ChCyV 2 remained in a separated branch indicating to be a divergent cyclovirus.

(iv) ***Anelloviridae***: Four gyrovirus-related contigs > 1,000 nt were recovered in this study (Fig S1a and Table S2, available in the online Supplementary Material). The first contig corresponds to a complete genome of chicken anemia virus (CAV); this shares 99% aa identity to VP1 protein of a CAV from China (GenBank no. ac. KU645522). The second contig represents the full genome of avian gyrovirus 2 (AGV2) with 99% identity to putative nucleocapsid of the prototype sequence (GenBank no. ac. NC_015396). Two other contigs matched to gyrovirus 4 (GyV4) and showed 97-99% identity to VP1 protein of a GyV4 recently reported in chicken faeces (GenBank no. ac. KY024580). Phylogenetic analysis of the VP1 nucleotide sequences confirmed the close relationship between gyroviruses reported here and its previously described counterparts (Fig. S1b).

(v) ***Reoviridae***: A total of twenty-six contigs related to members of the family *Reoviridae* were found (Table S2). These include members of three rotavirus species: Rotavirus D (15 contigs, ranging in size from 1,017 to 3,302 nt), Rotavirus F (9 contigs, 1,011 to 2,726 nt) and Rotavirus A (1 contig, 1,016 nt). The phylogenetic analysis of the VP2 nucleotide sequences confirms their species assignment (Fig. S2).

Another contig (1,157 nt-long) was detected which mapped to avian orthoreovirus (GenBank no. ac. KF741696), with 99% amino acid identity to lambda-A protein (Table S2). The phylogenetic analysis of such sequence supports its relatedness to avian orthoreoviruses (Fig. S3).

(vi) ***Picobirnaviridae***: Twenty-four contigs of picobirnavirus (PBVs) were assembled. Complete or nearly complete CDS encoding a capsid protein were generated in twelve contigs (Table S2). These sequences showed identity at the aa level ranging from 32 to 56% to capsid protein of PBVs of swine, dromedaries, otarines and humans (38-40). Other twelve contigs correspond to full or partial CDS of the RNA-dependent RNA polymerase (RdRp) protein and shared between 61 and 89% aa identity to the RdRp of PBVs identified in different mammals species (Fig 5a, Table S2).

Through phylogenetic analysis of RdRp sequences, the PBV genomes identified here were classified in genogroups I and II. In view of this, RdRp-contigs here reported were used for maximum-likelihood (ML) phylogenetic analyses, which revealed that only two sequences belong to PBV genogroup II. The additional ten RdRp-sequences clustered to genogroup I and were widely scattered in the tree presented in Fig. 5(b).

(vii) ***Astroviridae***: Nine contigs displaying similarity to the *Avastrovirus* genus, specifically to the species *Avian nephritis virus* (ANV) and *Chicken astrovirus* (CAstV) were detected. One complete ANV genome (RS/BR/15/6R, 6,890 nt-length) was recovered, with three open reading frames (ORFs) putatively coding for a nonstructural protein, a RdRp and a capsid protein (Fig. S4a). Such viral genome (ANV RS/BR/15/6R) showed 78% aa identity to the non-structural polyprotein of pigeon avian nephritis virus (GenBank no. ac. HQ889774). Two shorter sequences, corresponding to the partial CDS of ANV non-strutural and capsid proteins, were also identified (Table S2, Fig. S4a).

CAstV-related contigs (n=6) were 1,425 to 3,204 nt long and consist of full or partial CDS of capsid and non-structural proteins. The genetic maps of these sequences and the similarity to their counterpart are displayed in Table S2 and Fig. S4a.

Contigs showing identity to the capsid protein were selected to build a phylogenetic tree. The ML phylogenetic analysis supports the classification of the avastroviruses reported in this study at the species level (Fig. S4b).

(viii) *Caliciviridae*: A total of thirteen contigs of chicken calicivirus were found (Table S2). Among these contigs, two generated the complete genomes of novel chicken calicivirus (ChCaV RS/BR/15/1R-1 and 6R), resulting in 8,374 and 8,187-bases sequences, respectively (Fig. S5a). The genome organization of these contigs showed typical features of previously reported ChCaV genomes with a major ORF encoding the putative polyprotein and a minor ORF encoding the supposed VP2 protein. ML phylogenetic analysis based on the RdRp aa sequence revealed ChCaV RS/BR/15/1R-1 and 6R clustered in the proposed *Bavovirus* genus along with other ChCaV reported in Brazil (30), Fig S5(b).

(ix) *Parvoviridae*:

Aveparvovirus: Three contigs corresponding the full CDS of galliform aveparvovirus (GaPV/RS/BR/15/1R, 4R and 6S) genomes were assembled (Fig. 6a). The overall genomic organization of these sequences is similar to other GaPV, with three predicted ORFs, which encode the putative non-structural (NP1 and NS1) and capsid (VP1) proteins. In addition, GaPV/RS/BR/15/1R (5,265 nt-long) and 4R (5,256 nt) contain an inverted terminal repeat (ITR) sequences at both the right and left genomic termini. These sequence shared among 99 and 100% identity to NS1 of other GaPV previously described (Table S2). Three shorter contigs displaying incomplete coding region of GaPV were also found (Table S2).

Chapparvovirus: Three contigs showed higher similarity to “Protoparvovirus HK-2014” (GenBank ac.no. KM254174) recovered from chicken faeces, which has been recently

248 classified in the genus *Chapparvovirus*. Among these sequences, a 1,930 nt-long contig
249 (named chicken chapparvovirus, ChCPV 1 RS/BR/15/6S) contains a partial CDS of the
250 NS1 protein and shared 99% aa identity to KM254174. Two other contigs correspond to
251 the complete (ChCPV 2 RS/BR/15/2S, 4,432 nt in length) and partial (ChCPV 2
252 RS/BR/15 5S, 4,228 nt-long) coding regions of NS1 and capsid proteins, and showed 75
253 and 74% aa identity to NS1 of the KM254174 genome, respectively. The genomic
254 organization of these sequences is shown in Fig. 6a.

255 Dependoparvovirus: A near-complete genome comprising the full coding region of a
256 avian adeno-associated virus (AAAV/RS/BR/15/1R) were identified with 4,062 nt in
257 length and 94% aa identity to VP1 of the AAAV (GenBank ac.no.GQ368252) (Fig. 6a).

258 The ML phylogenetic analysis performed with NS1 nucleotide sequences from
259 parvoviruses described in this study confirmed their genus classification. Interestingly,
260 ChCPV 2 RS/BR/15/2S and 5S clustered on a separate branch, suggesting they form a
261 distinct lineage from the other two chapparvovirus identified in chickens (Fig. 6b).

262 **(x)** ***Adenoviridae***: Ten contigs > 1,000 nt matched to different regions of fowl
263 aviadenovirus (FAV) D (FAVD) genome. These contigs ranged from 1,017 to 2,422 nt
264 in length and displayed among 93 to 100% aa identity to other FAVD deposited in
265 GenBank as shown in Table S2. Sequence analysis of DNA polymerase confirms its close
266 relationship with other FAVD sequences (Fig. S6).

267 In addition, forty six contigs < 1,000 nt-long related to FAV E (FAVE) were found
268 (data not shown). These contigs were concatenated and used to measure the number of
269 matching reads.

270 **Disease association of different viruses**

In order to determine the numbers of reads corresponding to a specific virus, viral contigs were used to match reads in each pool. For this, contigs were taxonomically classified according to the best fitting hits. Next, very closely related contigs were concatenated and used to enumerate matching raw reads in samples from MAS-affected and healthy birds.

Significant differences among the distribution of viral reads matching the different eukaryotic viruses found in this study were compared using Mann-Whitney tests (Fig. 7). Higher numbers of viral reads were identified for megrivirus and sicinivirus (blue bars in Fig. 7) in MAS-affected group. Similarly, chicken-associated cyclovirus 2 (ChCyV 2), rotavirus D, astrovirus, galliform aveparvovirus, avian adeno-associated virus and fowl aviadenoviruses were more frequent in diseased chickens. In other hand, a greater amount of CRESS-DNA virus and ChCyV reads were found in healthy group, followed by gyroviruses, rotavirus F, chicken picobirnavirus and chicken calicivirus (red bars in Fig. 7). However, no statistical significance (p-value < 0.05) was reach for differences found between MAS-affected and healthy groups (Fig. 7).

In order to assess relatedness and to examine the overall taxonomic similarities between samples from MAS-affected and healthy groups, principal component analyses (PCA) and hierarchical clustering were performed. The PCA showed no clear separation between samples from both groups, but a considerable overlap between samples from MAS-affected and healthy birds (Fig. 8a). In agreement with PCA, the hierarchical clustering analysis on the MAS-affected and healthy groups revealed intermingled branches in the dendrogram. Additionally, a heat map data generated with the relative amount of reads did not reveal any distinct pattern that could be positively or negatively associated to either MAS-affected or healthy groups of birds (Fig. 8b).

DISCUSSION

296 Malabsorption syndrome is an economically important disease of young broilers
297 leading to inefficient food conversion, decreased production, immune dysfunctions and
298 elevated mortality rates (3). Clinically, the birds show decreased body weight, diarrhea
299 and defective feather development (1). So far, to the knowledge of the authors, viral
300 surveys in MAS-affected chickens have predominantly focused on PCR-based
301 approaches; such methodology is heavily dependent on previous knowledge of the
302 existing virus diversity; therefore, previously unidentified agents would remain as such
303 (8, 9). Here, metagenomics analyses, based on sequence-independent amplification and
304 deep sequencing on a high throughput platform, were used to investigate the role for
305 viruses in MAS.

306 Such approach allowed identification of a number of complete or partial genomes
307 of distinct representatives of putative members of a variety of viral families, not only in
308 samples from MAS-affected birds but also from healthy broilers.

309 The relative abundance of eukaryotic viral reads were compared between groups.
310 The genomes more often detected were representatives of nine known viral families.
311 Sequences of putative members of the family *Picornaviridae* were predominant in group
312 of diseased birds. However, no statistically significant differences were detected in the
313 amounts of reads detected in MAS-affected and healthy broilers (Fig. 7). Most of the
314 sequences related to picornaviruses corresponded to members of the genera *Megrivirus*
315 and *Sicinivirus*, with numerous distinct genotypes being detected. Three additional
316 contigs correspond to new genotypes of galliviruses and phacoviruses. Similar profiles
317 have been recently reported in faecal samples collected from birds with enteric disorders
318 and transmissible proventriculitis (41-45) as well as in healthy chickens (30, 46).

The second most common finding in both groups of birds were sequence reads that clustered along with CRESS-DNA viruses. These represent a rapidly growing, newly discovered and yet unassigned group of viruses (47). Although highly divergent, such small circular ssDNA viruses encode a well-conserved replication initiator protein (Rep) involved in rolling circle replication (48). Over the last decade, improvements in sequencing technologies have increased the number of known CRESS-DNA viruses detected from a wide variety of samples, including insects, plants, sewage and faeces from a wide range of vertebrates (30, 35, 36, 49-52). In this study, the number of reads of CRESS-DNA genomes showed no statistical difference in their relative contents when comparing MAS-affected and healthy birds. These findings are in agreement with the hypothesis that these agents may be commensals in the birds intestinal virome, although as has also been postulated, it is possible that such viruses might be acquired through food consumption (30, 47, 50, 53).

Contigs related to cyclovirus yielded five full genomes in this study. When comparing the amount of sequence reads of cycloviruses, here again no significant differences were found in frequencies of detection in MAS-affected and healthy animals. Although cycloviruses were first reported in stool samples from primates (54), cyclovirus genomes have been detected from a diversity of invertebrates and vertebrates specimens including insects (55), birds (56, 57) and mammals (57-61). Here, four contigs showed high degree of identity to a cyclovirus (Duck associated cyclovirus 1) detected in a cloacal swab from a wild mallard duck (56), with which it clustered, forming a monophyletic group. These findings may suggest cross-species transmission of cyclovirus from ducks to chickens, what would not be unreasonable. Another cyclovirus detected here, chicken associated cyclovirus 2 (ChCyV2), shared only 48 % identity to the Rep aa sequence from the dragonfly associated cyclovirus (55) and clustered into a highly supported, distinct

branch, suggesting that the ChCyV2 reported represents a novel species within the genus (61). However, assigning a host species may be challenging here, since this cyclovirus may be present in the birds'intestines in function of its diet, which might have included insects.

Reads corresponding to genomes of gyroviruses, members of *Anelloviridae* family, were also identified in both MAS-affected and healthy birds, although here again no association could be established between the number of reads detected in birds with or without MAS. The gyroviruses detected here could be classified in three species: CAV, AGV2 and GyV4. CAV is the causative agent of chicken infectious anemia and is distributed worldwide in commercial broiler flocks (62, 63). Likewise, numerous studies have reported AGV2 and GyV4 in chickens and/or mammals samples (meat, blood or faecal specimens) from different countries, suggesting the ubiquitous occurrence of these viruses (30, 64-66).

Sequences matching genomes of members of the *Reoviridae* included twenty-five contigs related to rotaviruses (RVs A, D and F) and one avian orthorevirus (ARV). In the present study, no significant differences were found in the number of reads of RVs and ARV when comparing MAS-affected and healthy animals. RVs are commonly associated with acute viral gastroenteritis in multiple animal species, affecting particularly the young (67). RV A is a well characterized virus and has been identified in both mammalian and avian species, often associated to enteric disorders (68, 69). RV D was first identified in chicken faeces (70) while RV F was detected in turkey and subsequently in broilers with runting-stunting syndrome (71). On its turn, ARVs are widespread and have been associated to MAS in 2-3 weeks old broilers, as well as to arthritis/tenosynovitis in older chickens (72). Although RVs and ARVs have been implicated in the aetiology of the runting-stunting syndrome of broiler chicks, they have also been detected in

369 asymptomatic birds (30, 73, 74), suggesting that these viruses can be part of normal
370 intestinal microbiota (75).

371 Picobirnavirus (PBVs) contigs generated twenty-four complete or nearly
372 complete CDS encoding the putative capsid (n=12) and the RdRp (n=12) proteins. Here
373 again no statistically significant differences could be detected between the numbers of
374 PBV reads in diseased or healthy birds. The classification of PBV genomes was based on the
375 *RdRp* gene; only two of those were classified in genogroup II. The others *RdRp* sequences
376 belonged to genogroup I, along with representatives of viruses detected in different
377 species, indicating that cross species transmission are probably frequent events among
378 these viruses (76, 77).

379 Avian astrovirus sequences yielded nine long contigs corresponding to complete
380 or partial genomes of previously unreported avian nephritis viruses (ANV; n=3) and
381 chicken astrovirus (CAstV; n=6) genotypes. Here, however, the distribution of avian
382 astrovirus related reads in diseased and healthy broilers showed no statistically significant
383 differences. Avian astroviruses have been reported in commercial poultry flocks
384 worldwide (5, 8, 78), occasionally associated with decreased growth rates and kidney
385 lesions in young chickens (79).

386 Regarding chicken caliciviruses, two complete genomes were identified here,
387 which where assigned in to the newly proposed genus *Bavovirus*, in the family
388 *Caliciviridae* (80). The chicken calicivirus genomes reported showed no significant
389 differences in the distribution of reads detected in MAS-affected or healthy birds. Chicken
390 caliciviruses were first reported in faecal samples from MAS-affected and healthy
391 broilers in Germany, also no association to disease (81). Therefore, the findings reported
392 here support the observations of those authors (81).

In relation to parvoviruses, once more the number of reads recovered from either diseased or healthy birds was not significantly different. The sequences of avian parvoviruses detected here could be classified within three genera, *Aveparvovirus*, *Chapparvovirus* and *Dependoparvovirus*. Six complete or partial CDS of *Galliform aveparvovirus* (GaPV) were identified. GaPV, also named chicken parvovirus, have previously been implicated in the development of MAS (82, 83), what was not supported in the present study. Other parvovirus sequences were assigned into the genus recently proposed genus *Chapparvovirus* (84). One of these sequences (ChCPV 1) showed high similarity to a new parvovirus discovered in chickens in South Korea, while two other contigs which were classified as ChCPV 2, shared only 74% and 75% identity to the latter. According to the ICTV criteria for species demarcation of the *Parvoviridae*, the ChCPV 2 genome here reported represents a new species within the *Chapparvovirus* genus (85). Another parvovirus contig identified clustered along genomes of the *Dependoparvovirus* genus, closely related to avian adeno-associated viruses.

Fowl adenovirus (FAV) D and E contigs were detected in low read numbers, and again with no statistically significant difference in the distribution of reads, in both diseased and healthy animals. FAV D and E infections are ubiquitous in chickens, where such agents are usually not associated to disease (86). More likely, such viruses are part of the normal intestinal flora of chickens.

In summary, when comparing the overall eukaryotic viral sequences detected in MAS-affected and healthy samples, no clear distinctive patterns could be associated to the occurrence of MAS in the virome profiles here obtained. These results support the hypothesis that there seems not to be a specific viral agent associated to the occurrence of MAS, as evidenced in this study. These findings point to the possibility that other

variables such as environmental factors, management conditions and perhaps other microorganisms may be related to the aetiology of MAS.

CONCLUDING REMARKS

In this study the faecal virome of MAS-affected and healthy chickens were analyzed comparatively. Genomes of a number of previously known and some as yet unreported viral genome sequences were identified in diseased and healthy birds. It was not possible to associate any particular virus or viruses with MAS, nor its relative abundancy (as revealed by the numbers of reads recovered) in diseased or healthy broilers. Future longitudinal studies may aid in attempting to define the aetiology of MAS.

MATERIAL AND METHODS

Biological samples

Samples were collected in 2015 (May to December) from seven different commercial poultry farms in Rio Grande do Sul State, Brazil. Seventy, 3-5 weeks old chickens (35 from MAS- affected birds; 35 from healthy ones) were collected. Five birds presenting clinical signs suggestive of MAS **(**retarded growth, apathy, diarrhea and defective feather development) were selected in each flock. Control samples were taken from five randomly collected, clinically healthy broilers, at the same age and from the same flocks. The average weight of MAS-affected birds was 0.89 kg; this represents 49,1% less than the average weight of healthy broilers (1.75 kg). The birds were euthanized, the intestinal tracts removed and frozen at −80 °C until processing. All procedures were performed in compliance with the Brazilian College of Animal Experimentation (COBEA) and approved by the Commission of Ethics on Animal Use of the Veterinary Research Institute Desidério Finamor (CEUA - IPVDF) – No. 21/2014.

Sample preparation, nucleic acids enrichment and high throughput sequencing

The total intestinal contents of the birds were processed in pools. From each of the seven farms, one pool comprising five stool samples from MAS-affected birds (named groups 1 to 7 “runt”, or G1 to G7 R). Equivalent pools were prepared from control birds, collected from the same flocks (named G1 to G7 “sound”, or G1 to G7 S). Each pool was comprised by one gram of each stool sample was diluted q.s.p. 10 ml in PBS (pH 7.2) and each five (collected from a same farm) were pooled. Such pools were vigorously vortexed for five minutes and clarified at 3,000 × g for 30 min at 4 °C. Subsequently, the supernatants were filtered through a 0.45 μm filter (Millipore) to remove bacteria and cell debris. These were then centrifuged at ~150,000 × g for 4 h at 4 °C (Sorvall AH629 rotor).

The resulting pellets were resuspended in ultrapure water and treated with 2 µL DNase (2 Units/µL, Turbo DNase, Ambion), 5 µL RNase A (20 mg/mL), Invitrogen) and 0,5 µL benzonase (25 Units/µL, Novagen). Such mixes were incubated at 37 °C for 2 h to digest nucleic acids unprotected by capsids (28). Viral RNA was extracted with TRIzol LS (Ambion) and reverse transcribed to generate cDNA libraries with a commercial kit (SeqPlexRNA Amplification Kit; Sigma Aldrich), following manufacturer's instructions. Viral DNA extraction was performed with a standard phenol-chloroform protocol (87) and enriched by multiple displacement amplification (MDA) with ϕ29 DNA polymerase (88).

The randomly enriched nucleic acids of each original pools were mixed in equimolar amounts (enriched cDNA plus enriched DNA) and purified with a DNA-cleaning kit (Genomic DNA Clean & Concentrator; Zymo Research). The quality and quantity of DNA preparations were determined in an L-Quant spectrophotometer (Loccus Biotechnology) and in a Qubit fluorometer (Invitrogen). For high throughput sequencing, DNA fragment libraries were prepared with 50 ng of the purified viral derived, enriched nucleic acids, using a Nextera DNA sample preparation kit (Illumina). Sequencing was performed in an Illumina®MiSeq instrument using the Illumina v2 reagent kit (2x250 paired-end reads). Supplementary data (sample identification and number of reads obtained from each library) are presented in Table S1.

Bioinformatics pipeline

Low-quality sequencing reads with a Phred quality score < 20 were trimmed using PRINSEQ version 0.20.4 (89). The paired-end sequence reads were *de novo* assembled in contigs using metaSPAdes 3.10.1 (90). The assembled contigs and singlet sequences were analysed by BLASTx against a viral protein database. Sequences with the best

BLAST scores (E values of $\leq 10^{-3}$) were selected and assigned into known viral families and, where applicable, to the respective CRESS-DNA current classification. Contigs > 1,000 nt were confirmed by mapping reads and selected to perform ORF predictions and genome annotations using the Geneious software (version 9.1.8). For the purposes of the present study and for sake of brevity, only contigs >1000 nt are described, with the exception of those of fowl adenovirus E, for which no contigs >1000 nt were detected).

Assessment of viral abundance

Numbers of raw reads matching selected viral sequences were measured with the Geneious software with all raw data output reads in medium-low sensitivity/fast mode. The amount of virus-specific reads detected in individual samples were divided by the total number of reads generated from each sample. The read numbers, were expressed as percentages of viral reads.

Significant differences among the distribution of sequences matching viral contigs in MAS-affected and healthy groups were compared using Mann-Whitney tests (GraphPad Prism software version 5). In order to examine overall taxonomic similarities between metagenomes, principal components analysis (PCA) was applied (IBM SPSS Statistics software version 22). As a confirmatory visualization, hierarchical clustering and heat map analyses were performed using the Gplots package in the RStudio software.

Phylogenetic analyses

Sequences representative of known viral families, as well as CRESS-DNA genomes, were obtained from GenBank and then aligned with the sequences identified in the present study using either MUSCLE or MAFFT software (91, 92). These were used to generate maximum-likelihood phylogenetic trees using PHYML (93) with best fit substitution models determined by Smart Model Selection (94). Statistical significance

499 analyses of tree topologies were performed with the approximate likelihood branch
500 support test (aLRT) (95).

501 **Nucleotide sequences accession numbers**

502 Complete or partial viral genome sequences identified in this study were
503 submitted to GenBank under the accession numbers presented in Table S2.

504 **Funding information**

505 Work supported by the FINEP (proj. SANIMARS no. 01.10.0783.04 and proj.
506 MAIS ÁGUA no. 0112.0113), CNPq and CAPES. DAL has been supported by a CAPES
507 Ph.D. grant. PMR is a CNPq 1A research fellow.

508 **Conflicts of interest**

509 The authors declare no conflicts of interest regarding this manuscript.

510

References

1. Songserm T, Zekarias B, van Roozelaar DJ, Kok RS, Pol JM, Pijpers AA, et al. Experimental reproduction of malabsorption syndrome with different combinations of reovirus, Escherichia coli, and treated homogenates obtained from broilers. Avian diseases. 2002;46(1):87-94.
2. Sellers H, Linneman E, Icard AH, Mundt E. A purified recombinant baculovirus expressed capsid protein of a new astrovirus provides partial protection to runting-stunting syndrome in chickens. Vaccine. 2010;28(5):1253-63.
3. Day JM, Zsak L. Recent progress in the characterization of avian enteric viruses. Avian diseases. 2013;57(3):573-80.
4. Zsak L, Cha RM, Day JM. Chicken parvovirus-induced runting-stunting syndrome in young broilers. Avian diseases. 2013;57(1):123-7.
5. Day JM, Ballard LL, Duke MV, Scheffler BE, Zsak L. Metagenomic analysis of the turkey gut RNA virus community. Virology journal. 2010;7:313.
6. Kang KI, El-Gazzar M, Sellers HS, Dorea F, Williams SM, Kim T, et al. Investigation into the aetiology of runting and stunting syndrome in chickens. Avian pathology : journal of the WVPA. 2012;41(1):41-50.
7. Baxendale W, Mebatsion T. The isolation and characterisation of astroviruses from chickens. Avian pathology : journal of the WVPA. 2004;33(3):364-70.
8. Pantin-Jackwood MJ, Day JM, Jackwood MW, Spackman E. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian diseases. 2008;52(2):235-44.
9. Pantin-Jackwood MJ, Strother KO, Mundt E, Zsak L, Day JM, Spackman E. Molecular characterization of avian astroviruses. Archives of virology. 2011;156(2):235-44.
10. Day JM, Zsak L. Determination and analysis of the full-length chicken parvovirus genome. Virology. 2010;399(1):59-64.
11. Koo BS, Lee HR, Jeon EO, Han MS, Min KC, Lee SB, et al. Genetic characterization of three novel chicken parvovirus strains based on analysis of their coding sequences. Avian pathology : journal of the WVPA. 2015;44(1):28-34.
12. Zsak L, Strother KO, Day JM. Development of a polymerase chain reaction procedure for detection of chicken and turkey parvoviruses. Avian diseases. 2009;53(1):83-8.
13. Zsak L, Strother KO, Kisary J. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian pathology : journal of the WVPA. 2008;37(4):435-41.
14. Chen WT, Wu YL, Chen T, Cheng CS, Chan HL, Chou HC, et al. Proteomics analysis of the DF-1 chicken fibroblasts infected with avian reovirus strain S1133. PloS one. 2014;9(3):e92154.
15. Songserm T, Pol JM, van Roozelaar D, Kok GL, Wagenaar F, ter Huurne AA. A comparative study of the pathogenesis of malabsorption syndrome in broilers. Avian diseases. 2000;44(3):556-67.
16. Otto P, Liebler-Tenorio EM, Elschner M, Reetz J, Lohren U, Diller R. Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). Avian diseases. 2006;50(3):411-8.
17. Spackman E, Day JM, Pantin-Jackwood MJ. Astrovirus, reovirus, and rotavirus concomitant infection causes decreased weight gain in broad-breasted white poults. Avian diseases. 2010;54(1):16-21.
18. Devaney R, Trudgett J, Trudgett A, Meharg C, Smyth V. A metagenomic comparison of endemic viruses from broiler chickens with runting-stunting syndrome and from normal birds. Avian pathology : journal of the WVPA. 2016;45(6):616-29.
19. Palade EA, Kisary J, Benyeda Z, Mandoki M, Balka G, Jakab C, et al. Naturally occurring parvoviral infection in Hungarian broiler flocks. Avian pathology : journal of the WVPA. 2011;40(2):191-7.

561 20. Bexfield N, Kellam P. Metagenomics and the molecular identification of novel viruses.
562 Veterinary journal. 2011;190(2):191-8.
563 21. Rosario K, Breitbart M. Exploring the viral world through metagenomics. Current opinion
564 in virology. 2011;1(4):289-97.
565 22. Cheung AK, Ng TF, Lager KM, Alt DP, Delwart E, Pogranichniy RM. Identification of
566 several clades of novel single-stranded circular DNA viruses with conserved stem-loop structures
567 in pig feces. Archives of virology. 2015;160(1):353-8.
568 23. Cheung AK, Ng TF, Lager KM, Bayles DO, Alt DP, Delwart EL, et al. A divergent clade of
569 circular single-stranded DNA viruses from pig feces. Archives of virology. 2013;158(10):2157-62.
570 24. Day JM, Oakley BB, Seal BS, Zsak L. Comparative analysis of the intestinal bacterial and
571 RNA viral communities from sentinel birds placed on selected broiler chicken farms. PloS one.
572 2015;10(1).
573 25. Kim HK, Park SJ, Nguyen VG, Song DS, Moon HJ, Kang BK, et al. Identification of a novel
574 single-stranded, circular DNA virus from bovine stool. The Journal of general virology. 2012;93(Pt
575 3):635-9.
576 26. Ng TF, Kondov NO, Deng X, Van Eenennaam A, Neibergs HL, Delwart E. A metagenomics
577 and case-control study to identify viruses associated with bovine respiratory disease. Journal of
578 virology. 2015;89(10):5340-9.
579 27. Shah JD, Baller J, Zhang Y, Silverstein K, Xing Z, Cardona CJ. Comparison of tissue sample
580 processing methods for harvesting the viral metagenome and a snapshot of the RNA viral
581 community in a turkey gut. Journal of virological methods. 2014;209:15-24.
582 28. Shan T, Li L, Simmonds P, Wang C, Moeser A, Delwart E. The fecal virome of pigs on a
583 high-density farm. Journal of virology. 2011;85(22):11697-708.
584 29. Zhang B, Tang C, Yue H, Ren Y, Song Z. Viral metagenomics analysis demonstrates the
585 diversity of viral flora in piglet diarrhoeic faeces in China. The Journal of general virology.
586 2014;95(Pt 7):1603-11.
587 30. Lima DA, Cibulski SP, Finkler F, Teixeira TF, Varela APM, Cerva C, et al. Faecal virome of
588 healthy chickens reveals a large diversity of the eukaryote viral community, including novel
589 circular ssDNA viruses. The Journal of general virology. 2017;98(4):690-703.
590 31. Cox-Foster DL, Conlan S, Holmes EC, Palacios G, Evans JD, Moran NA, et al. A
591 metagenomic survey of microbes in honey bee colony collapse disorder. Science.
592 2007;318(5848):283-7.
593 32. Mihalov-Kovacs E, Feher E, Martella V, Banyai K, Farkas SL. The fecal virome of
594 domesticated animals. Virusdisease. 2014;25(2):150-7.
595 33. Victoria JG, Kapoor A, Dupuis K, Schnurr DP, Delwart EL. Rapid identification of known
596 and new RNA viruses from animal tissues. PLoS pathogens. 2008;4(9):e1000163.
597 34. Victoria JG, Kapoor A, Li L, Blinkova O, Slikas B, Wang C, et al. Metagenomic analyses of
598 viruses in stool samples from children with acute flaccid paralysis. Journal of virology.
599 2009;83(9):4642-51.
600 35. Krupovic M, Ghabrial SA, Jiang D, Varsani A. Genomoviridae: a new family of widespread
601 single-stranded DNA viruses. Archives of virology. 2016;161(9):2633-43.
602 36. Ng TFF, Zhang W, Sachsenröder J, Kondov NO, da Costa AC, Vega E, et al. A diverse group
603 of small circular ssDNA viral genomes in human and non-human primate stools. Virus Evolution.
604 2015;1(1):vev017.
605 37. Altan E, Del Valle Mendoza J, Deng X, Phan TG, Sadeghi M, Delwart EL. Small Circular
606 Rep-Encoding Single-Stranded DNA Genomes in Peruvian Diarrhea Virome. Genome
607 announcements. 2017;5(38).
608 38. Banyai K, Potgieter C, Gellert A, Ganesh B, Tempesta M, Lorusso E, et al. Genome
609 sequencing identifies genetic and antigenic divergence of porcine picobirnaviruses. The Journal
610 of general virology. 2014;95(Pt 10):2233-9.

611 39. Woo PC, Lau SK, Teng JL, Tsang AK, Joseph M, Wong EY, et al. Metagenomic analysis of
612 viromes of dromedary camel fecal samples reveals large number and high diversity of
613 circoviruses and picobirnaviruses. Virology. 2014;471-473:117-25.
614 40. Woo PC, Teng JL, Bai R, Wong AY, Martelli P, Hui SW, et al. High Diversity of Genogroup
615 I Picobirnaviruses in Mammals. Frontiers in microbiology. 2016;7:1886.
616 41. Boros A, Pankovics P, Adonyi A, Fenyvesi H, Day JM, Phan TG, et al. A diarrheic chicken
617 simultaneously co-infected with multiple picornaviruses: Complete genome analysis of avian
618 picornaviruses representing up to six genera. Virology. 2016;489:63-74.
619 42. Boros A, Pankovics P, Knowles NJ, Nemes C, Delwart E, Reuter G. Comparative complete
620 genome analysis of chicken and Turkey megriviruses (family picornaviridae): long 3' untranslated
621 regions with a potential second open reading frame and evidence for possible recombination.
622 Journal of virology. 2014;88(11):6434-43.
623 43. Bullman S, Kearney K, O'Mahony M, Kelly L, Whyte P, Fanning S, et al. Identification and
624 genetic characterization of a novel picornavirus from chickens. The Journal of general virology.
625 2014;95(Pt 5):1094-103.
626 44. Kim HR, Yoon SJ, Lee HS, Kwon YK. Identification of a picornavirus from chickens with
627 transmissible viral proventriculitis using metagenomic analysis. Archives of virology.
628 2015;160(3):701-9.
629 45. Zhou H, Zhu S, Quan R, Wang J, Wei L, Yang B, et al. Identification and Genome
630 Characterization of the First Sicinivirus Isolate from Chickens in Mainland China by Using Viral
631 Metagenomics. PloS one. 2015;10(10):e0139668.
632 46. Boros A, Nemes C, Pankovics P, Kapusinszky B, Delwart E, Reuter G. Genetic
633 characterization of a novel picornavirus in turkeys (Meleagris gallopavo) distinct from turkey
634 galliviruses and megriviruses and distantly related to the members of the genus Avihepatovirus.
635 The Journal of general virology. 2013;94(Pt 7):1496-509.
636 47. Rosario K, Dayaram A, Marinov M, Ware J, Kraberger S, Stainton D, et al. Diverse circular
637 ssDNA viruses discovered in dragonflies (Odonata: Epiprocta). The Journal of general virology.
638 2012;93(Pt 12):2668-81.
639 48. Rosario K, Duffy S, Breitbart M. A field guide to eukaryotic circular single-stranded DNA
640 viruses: insights gained from metagenomics. Archives of virology. 2012;157(10):1851-71.
641 49. Kraberger S, Arguello-Astorga GR, Greenfield LG, Galilee C, Law D, Martin DP, et al.
642 Characterisation of a diverse range of circular replication-associated protein encoding DNA
643 viruses recovered from a sewage treatment oxidation pond. Infection, genetics and evolution :
644 journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2015;31:73-
645 86.
646 50. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U, Fan Ng TF, et al. Small circular
647 single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in
648 untreated sewage. Virology. 2015;482:98-104.
649 51. Reuter G, Boros A, Delwart E, Pankovics P. Novel circular single-stranded DNA virus from
650 turkey faeces. Archives of virology. 2014;159(8):2161-4.
651 52. Steel O, Kraberger S, Sikorski A, Young LM, Catchpole RJ, Stevens AJ, et al. Circular
652 replication-associated protein encoding DNA viruses identified in the faecal matter of various
653 animals in New Zealand. Infection, genetics and evolution : journal of molecular epidemiology
654 and evolutionary genetics in infectious diseases. 2016;43:151-64.
655 53. Delwart E, Li L. Rapidly expanding genetic diversity and host range of the Circoviridae
656 viral family and other Rep encoding small circular ssDNA genomes. Virus research. 2012;164(1-
657 2):114-21.
658 54. Li L, Kapoor A, Slikas B, Bamidele OS, Wang C, Shaukat S, et al. Multiple diverse
659 circoviruses infect farm animals and are commonly found in human and chimpanzee feces.
660 Journal of virology. 2010;84(4):1674-82.

55. Dayaram A, Potter KA, Moline AB, Rosenstein DD, Marinov M, Thomas JE, et al. High global diversity of cycloviruses amongst dragonflies. The Journal of general virology. 2013;94(Pt 8):1827-40.
56. Feher E, Kaszab E, Forro B, Bali K, Marton S, Lengyel G, et al. Genome sequence of a mallard duck origin cyclovirus, DuACyV-1. Archives of virology. 2017;162(12):3925-9.
57. Li L, Shan T, Soji OB, Alam MM, Kunz TH, Zaidi SZ, et al. Possible cross-species transmission of circoviruses and cycloviruses among farm animals. The Journal of general virology. 2011;92(Pt 4):768-72.
58. Li L, Giannitti F, Low J, Keyes C, Ullmann LS, Deng X, et al. Exploring the virome of diseased horses. The Journal of general virology. 2015;96(9):2721-33.
59. Lima FE, Cibulski SP, Dos Santos HF, Teixeira TF, Varela AP, Roehe PM, et al. Genomic characterization of novel circular ssDNA viruses from insectivorous bats in Southern Brazil. PloS one. 2015;10(2):e0118070.
60. Male MF, Kraberger S, Stainton D, Kami V, Varsani A. Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific flying fox (Pteropus tonganus) faeces. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases. 2016;39:279-92.
61. Rosario K, Breitbart M, Harrach B, Segales J, Delwart E, Biagini P, et al. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. Archives of virology. 2017;162(5):1447-63.
62. Eltahir YM, Qian K, Jin W, Wang P, Qin A. Molecular epidemiology of chicken anemia virus in commercial farms in China. Virology journal. 2011;8:145.
63. Olszewska-Tomczyk M, Swieton E, Minta Z, Smietanka K. Occurrence and Phylogenetic Studies of Chicken Anemia Virus from Polish Broiler Flocks. Avian diseases. 2016;60(1):70-4.
64. Chu DK, Poon LL, Chiu SS, Chan KH, Ng EM, Bauer I, et al. Characterization of a novel gyrovirus in human stool and chicken meat. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2012;55(3):209-13.
65. dos Santos HF, Knak MB, de Castro FL, Slongo J, Ritterbusch GA, Klein TA, et al. Variants of the recently discovered avian gyrovirus 2 are detected in Southern Brazil and The Netherlands. Veterinary microbiology. 2012;155(2-4):230-6.
66. Rijsewijk FA, Dos Santos HF, Teixeira TF, Cibulski SP, Varela AP, Dezen D, et al. Discovery of a genome of a distant relative of chicken anemia virus reveals a new member of the genus Gyrovirus. Archives of virology. 2011;156(6):1097-100.
67. Pauly M, Oni OO, Sausy A, Owoade AA, Adeyefa CAO, Muller CP, et al. Molecular epidemiology of Avian Rotaviruses Group A and D shed by different bird species in Nigeria. Virology journal. 2017;14(1):111.
68. Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. Veterinary microbiology. 2010;140(3-4):246-55.
69. Matthijnssens J, Van Ranst M. Genotype constellation and evolution of group A rotaviruses infecting humans. Current opinion in virology. 2012;2(4):426-33.
70. Pedley S, Bridger JC, Chasey D, McCrae MA. Definition of two new groups of atypical rotaviruses. The Journal of general virology. 1986;67 (Pt 1):131-7.
71. Otto PH, Ahmed MU, Hotzel H, Machnowska P, Reetz J, Roth B, et al. Detection of avian rotaviruses of groups A, D, F and G in diseased chickens and turkeys from Europe and Bangladesh. Veterinary microbiology. 2012;156(1-2):8-15.
72. Farkas SL, Marton S, Dandar E, Kugler R, Gal B, Jakab F, et al. Lineage diversification, homo- and heterologous reassortment and recombination shape the evolution of chicken orthoreoviruses. Scientific reports. 2016;6:36960.
73. Chen F, Knutson TP, Porter RE, Ciarlet M, Mor SK, Marthaler DG. Genome characterization of Turkey Rotavirus G strains from the United States identifies potential recombination events with human Rotavirus B strains. The Journal of general virology. 2017;98(12):2931-6.

713 74. Moura-Alvarez J, Chacon JV, Scanavini LS, Nunez LF, Astolfi-Ferreira CS, Jones RC, et al.
714 Enteric viruses in Brazilian turkey flocks: single and multiple virus infection frequency according
715 to age and clinical signs of intestinal disease. Poultry science. 2013;92(4):945-55.
716 75. Shah JD, Desai PT, Zhang Y, Scharber SK, Baller J, Xing ZS, et al. Development of the
717 Intestinal RNA Virus Community of Healthy Broiler Chickens. PloS one. 2016;11(2):e0150094.
718 76. Giordano MO, Martinez LC, Masachessi G, Barril PA, Ferreyra LJ, Isa MB, et al. Evidence
719 of closely related picobirnavirus strains circulating in humans and pigs in Argentina. The Journal
720 of infection. 2011;62(1):45-51.
721 77. Kapusinszky B, Ardeshir A, Mulvaney U, Deng X, Delwart E. Case-Control Comparison of
722 Enteric Viromes in Captive Rhesus Macaques with Acute or Idiopathic Chronic Diarrhea. Journal
723 of virology. 2017;91(18).
724 78. Espinoza LL, Beserra LA, Soares RM, Gregori F. Avian nephritis virus (ANV) on Brazilian
725 chickens farms: circulating genotypes and intra-genotypic diversity. Archives of virology.
726 2016;161(12):3455-62.
727 79. Zhao W, Hua XG, Yuan L, Cui L, Shan TL, Dai XQ, et al. Sequence analyses of the
728 representative Chinese-prevalent strain of avian nephritis virus in healthy chicken flocks. Avian
729 diseases. 2011;55(1):65-9.
730 80. Wolf S, Reetz J, Hoffmann K, Grundel A, Schwarz BA, Hanel I, et al. Discovery and genetic
731 characterization of novel caliciviruses in German and Dutch poultry. Archives of virology.
732 2012;157(8):1499-507.
733 81. Wolf S, Reetz J, Otto P. Genetic characterization of a novel calicivirus from a chicken.
734 Archives of virology. 2011;156(7):1143-50.
735 82. Finkler F, Lima DA, Cerva C, Cibulski SP, Teixeira TF, Dos Santos HF, et al. Chicken
736 parvovirus viral loads in cloacal swabs from malabsorption syndrome-affected and healthy
737 broilers. Tropical animal health and production. 2016;48(8):1685-9.
738 83. Finkler F, Lima DA, Cerva C, Moraes LB, Cibulski SP, Teixeira TF, et al. Chicken parvovirus
739 and its associations with malabsorption syndrome. Research in veterinary science.
740 2016;107:178-81.
741 84. Yang S, Liu Z, Wang Y, Li W, Fu X, Lin Y, et al. A novel rodent Chapparvovirus in feces of
742 wild rats. Virology journal. 2016;13:133.
743 85. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, et al. The
744 family Parvoviridae. Archives of virology. 2014;159(5):1239-47.
745 86. Domanska-Blicharz K, Tomczyk G, Smietanka K, Kozaczynski W, Minta Z. Molecular
746 characterization of fowl adenoviruses isolated from chickens with gizzard erosions. Poultry
747 science. 2011;90(5):983-9.
748 87. Sambrook J, Russel DW. Molecular cloning: a laboratory manual. 3ed, editor. New York,
749 USA: Cold Spring Harbor; 2001.
750 88. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, et al. Comprehensive human
751 genome amplification using multiple displacement amplification. Proceedings of the National
752 Academy of Sciences of the United States of America. 2002;99(8):5261-6.
753 89. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets.
754 Bioinformatics. 2011;27(6):863-4.
755 90. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile
756 metagenomic assembler. Genome research. 2017;27(5):824-34.
757 91. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high
758 throughput. Nucleic acids research. 2004;32(5):1792-7.
759 92. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7:
760 improvements in performance and usability. Molecular biology and evolution. 2013;30(4):772-
761 80.
762 93. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms
763 and methods to estimate maximum-likelihood phylogenies: assessing the performance of
764 PhyML 3.0. Systematic biology. 2010;59(3):307-21.

765 94. Lefort V, Longueville JE, Gascuel O. SMS: Smart Model Selection in PhyML. Molecular
766 biology and evolution. 2017;34(9):2422-4.
767 95. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large
768 phylogenies by maximum likelihood. Systematic biology. 2003;52(5):696-704.

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Fig.1. Classification of sequence reads detected in stool samples from (a) MAS-affected and (b) healthy chickens. Pie charts represent relative amounts of reads classified by different eukaryotic viral families in faecal samples from MAS-affected and healthy chickens.

Fig. 2. Putative genome organization and phylogenetic analysis of picornavirus genomes identified in faeces of MAS-affected and healthy chickens. (a) Schematic representation of the prototypic genome of chicken sicinivirus (NC_028380). Grey bars represent the coverage of genomes of megriviruses, siciniviruses, phacoviruses and galliviruses contigs identified in this study. Only contigs >1000 nts are represented. (b) Maximum likelihood phylogenetic tree reconstructed with the substitution model LG of 3D polymerase amino acid sequences (dark shading) from representative members of the *Picornaviridae* family. The branch support is evidenced only for the clades with an aLRT support greater than 70 %.The picornaviruses recovered in this study are shown in bold.

Fig. 3. Genome organization of CRESS-DNA viruses and cycloviruses identified in faeces of MAS-affected and healthy chickens. (a) Putative genome maps of gemycircularvirus, smacoviruses and chicken stool associated circular viruses. (b) Putative genome organization of chicken associated cycloviruses.

Fig. 4. Maximum likelihood phylogenetic tree reconstructed with the substitution model VT+I+G on Rep aa sequences from different CRESS-DNA viruses. The branch support is evidenced only for the clades with an aLRT greater than 70 %. The CRESS-DNA viruses and cycloviruses identified here are highlighted in bold.

Fig. 5. Genomic organization and phylogenetic analysis of chicken picobirnaviruses genomes based on the nucleotide sequences coding for RdRp. (a) Schematic representation of the ChPBV-contigs recovered from faeces of MAS-affected and healthy chickens. (b) Phylogenetic inferences carried out by maximum likelihood phylogenetic analysis with the GTR+G+I substitution model. Branch support is highlighted only for clades with an aLRT greater than 70 %. The ChPBV genomes reported here are shown in bold.

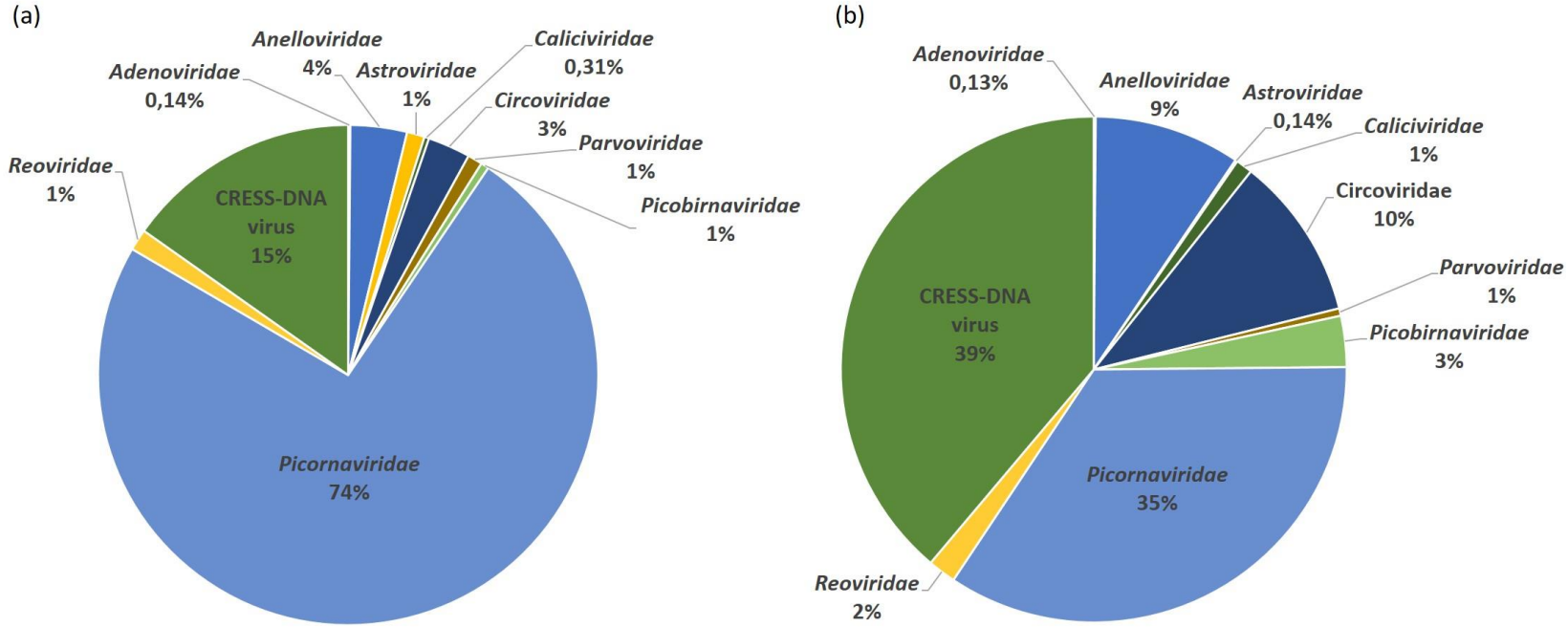
Fig. 6. Schematic representation and phylogenetic analysis of parvovirus genomes. (a) Putative genome organization of the parvovirus related contigs. (b) Phylogenetic tree based on the NS1 nucleotide sequences from representative members of subfamily *Parvovirinae*. Inferences carried out by maximum likelihood phylogenetic analysis with GTR+G+I substitution model. The branch support is evidenced only for the clades with an aLRT greater than 70 %. The parvoviruses recovered here are shown in bold.

Fig. 7. Statistical analysis of the distribution of numbers of reads matching different eukaryotic viruses in MAS-affected and healthy birds. The mean difference* reflect the average of reads from MAS-affected group minus the average of healthy group. The color bars are group mean differences and represent higher (blue) or lower (red) read numbers in MAS-affected or healthy chickens. Mann-Whitney test p-values indicate whether the red or blue values are statistically significant (p>0,05).

Fig. 8. Principal component analysis (PCA) and hierarchical clustering of MAS-affected and healthy animal groups. (a) PCA from pooled samples of birds showing clinical signs of MAS (red circles) and healthy chickens (blue diamonds). (b) Hierarchical clustering of the number of reads matching eukaryotic viruses in each sample The columns represent the analyzed samples (G1-G7R and G1-G7S), while the rows represent the distribution

of reads of each specific virus. The clustering tree of samples is shown at the top. The white color shows the highest relative amount of reads while the red color indicates the least amount of reads matching to each viruses.

822 Fig. 1



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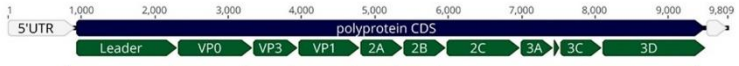
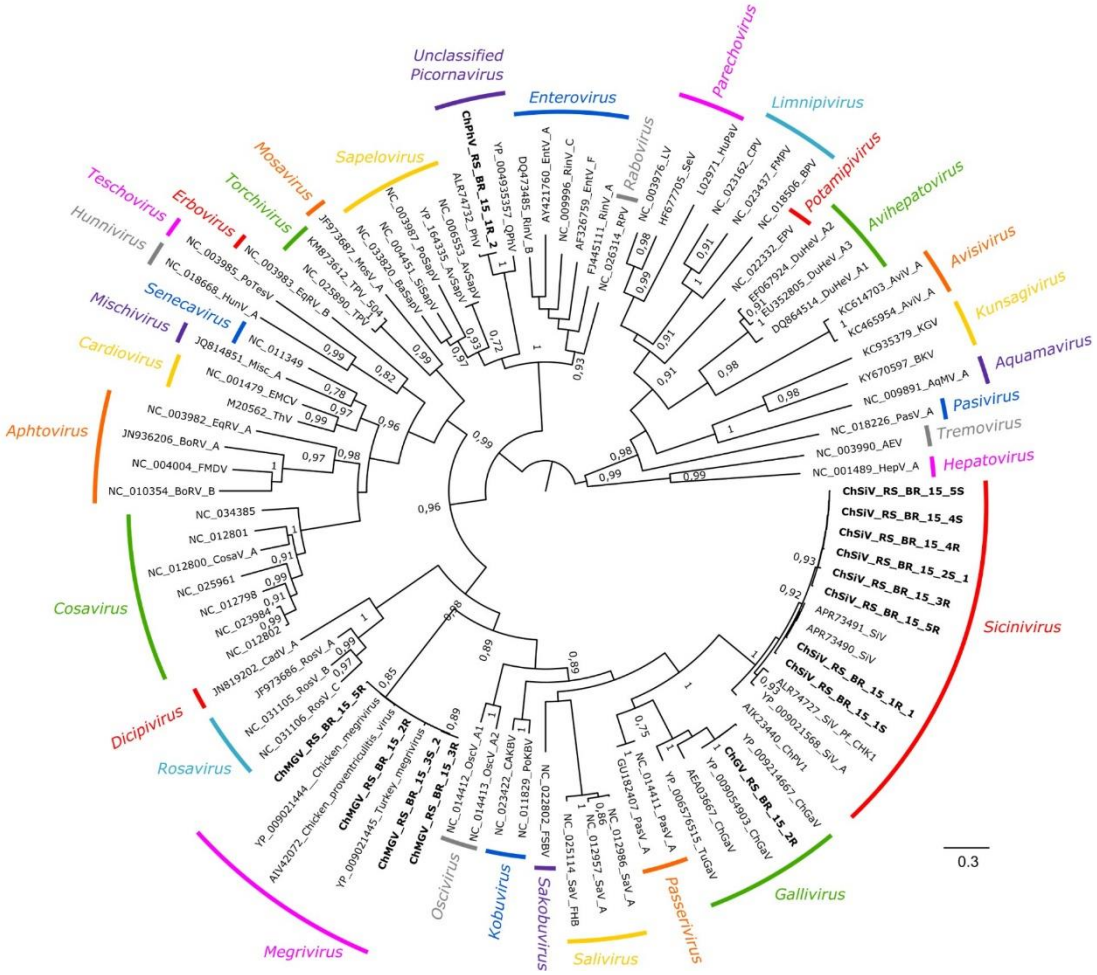
825 Fig. 2

(a)



Virus	Genotype	5'UTR	Polyprotein CDS: Leader	VP0	VP3	VP1	2A	2B	2C	3A	3C	3D	3'UTR
Chicken megrivirus	RS/BR/15/2R												
Chicken megrivirus	RS/BR/15/3R												
Chicken megrivirus	RS/BR/15/5R												
Chicken megrivirus	RS/BR/15/3S-2												
Chicken sicinivirus	RS/BR/15/1R-1												
Chicken sicinivirus	RS/BR/15/3R												
Chicken sicinivirus	RS/BR/15/4R												
Chicken sicinivirus	RS/BR/15/5R												
Chicken sicinivirus	RS/BR/15/7S												
Chicken sicinivirus	RS/BR/15/2S-1												
Chicken sicinivirus	RS/BR/15/4S												
Chicken sicinivirus	RS/BR/15/5S												
Chicken phacovirus	RS/BR/15/1R-1												
Chicken gallivirus	RS/BR/15/2R												

(b)



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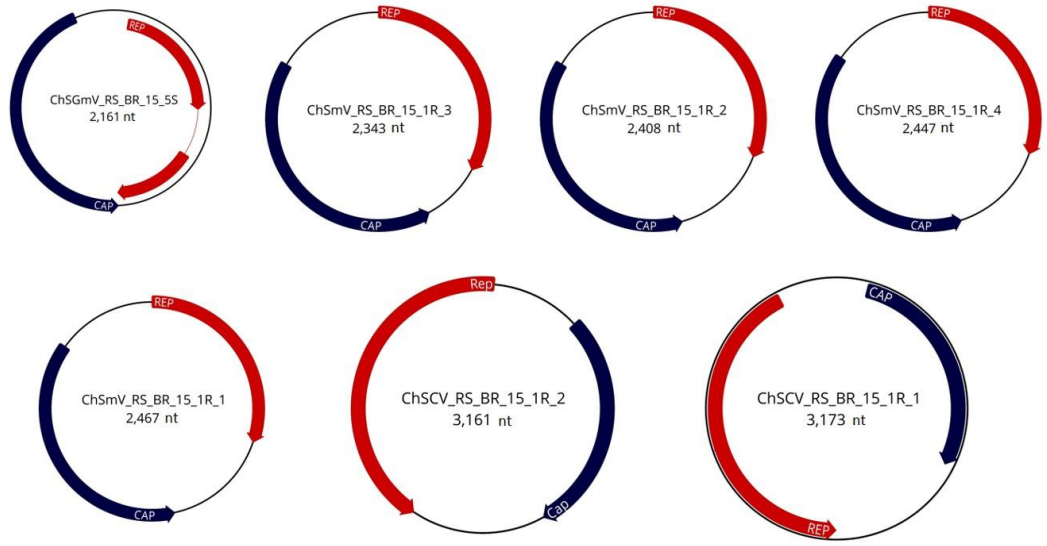
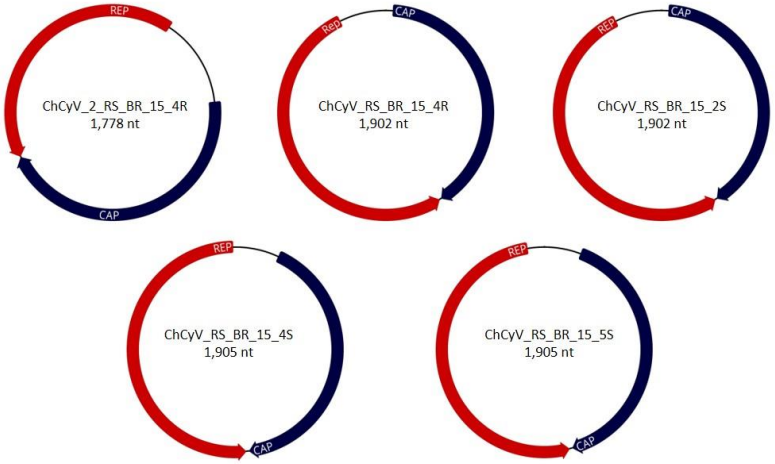
828 Fig. 3

(a)



(b)



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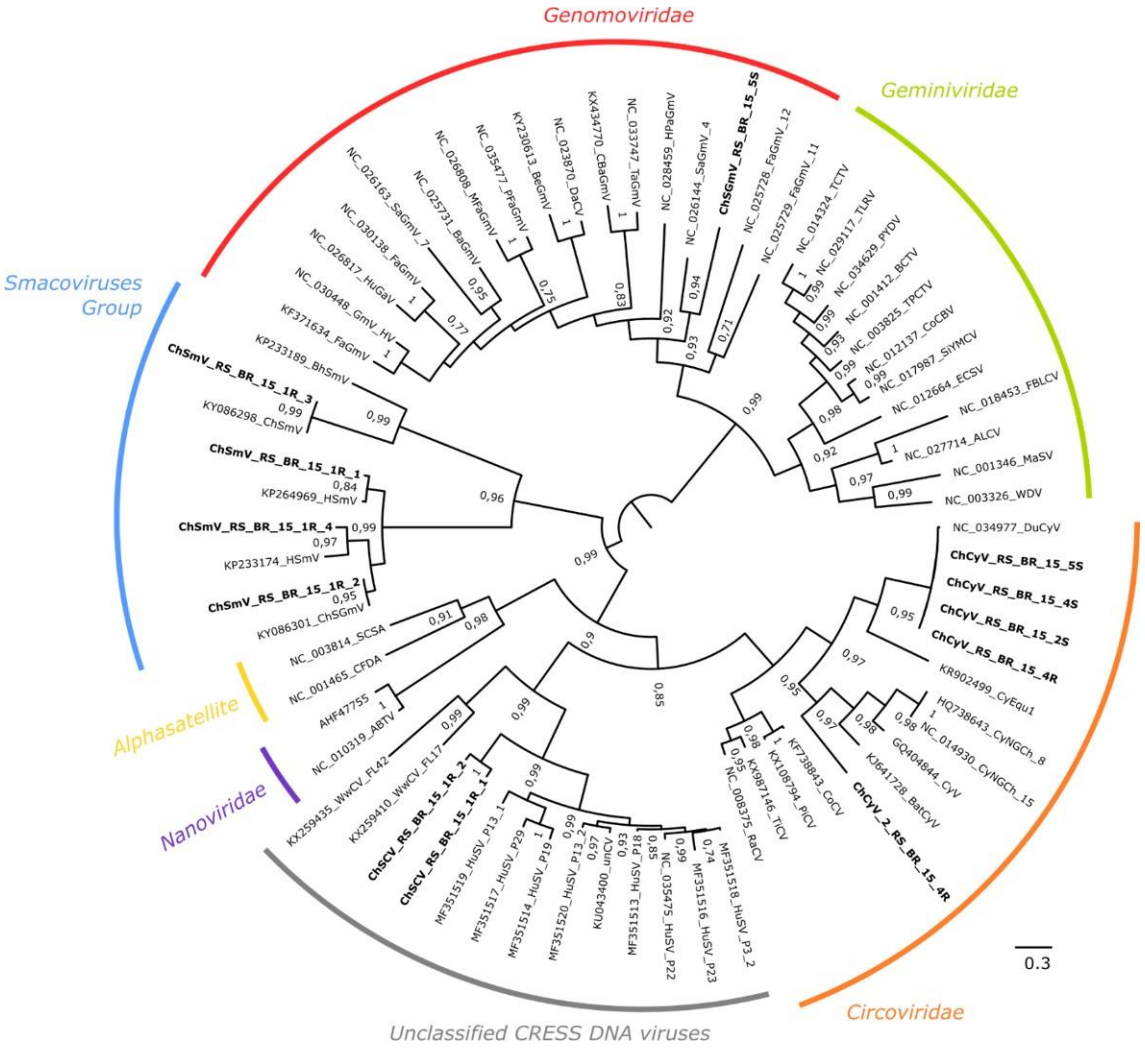
831 Fig. 4



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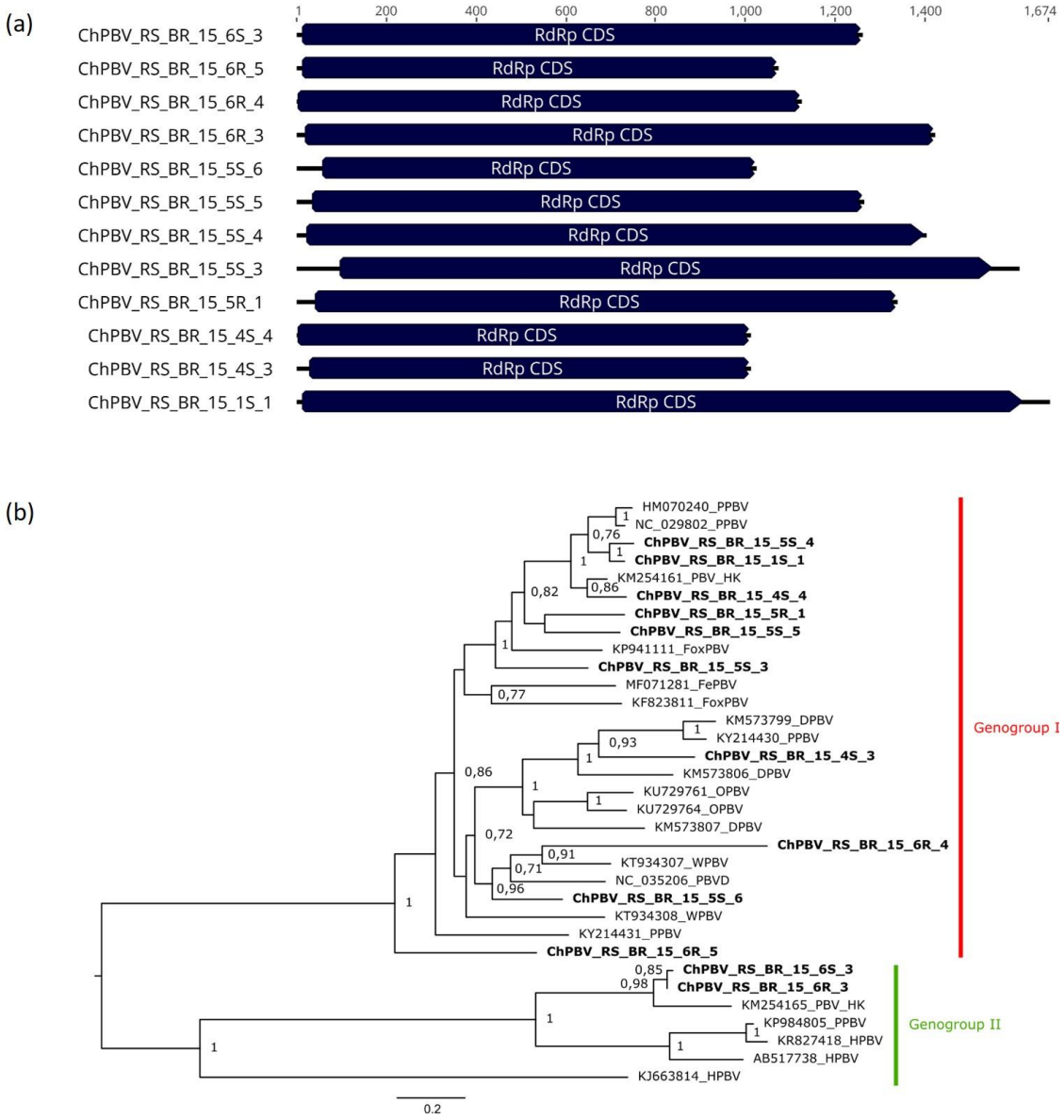
834 Fig. 5



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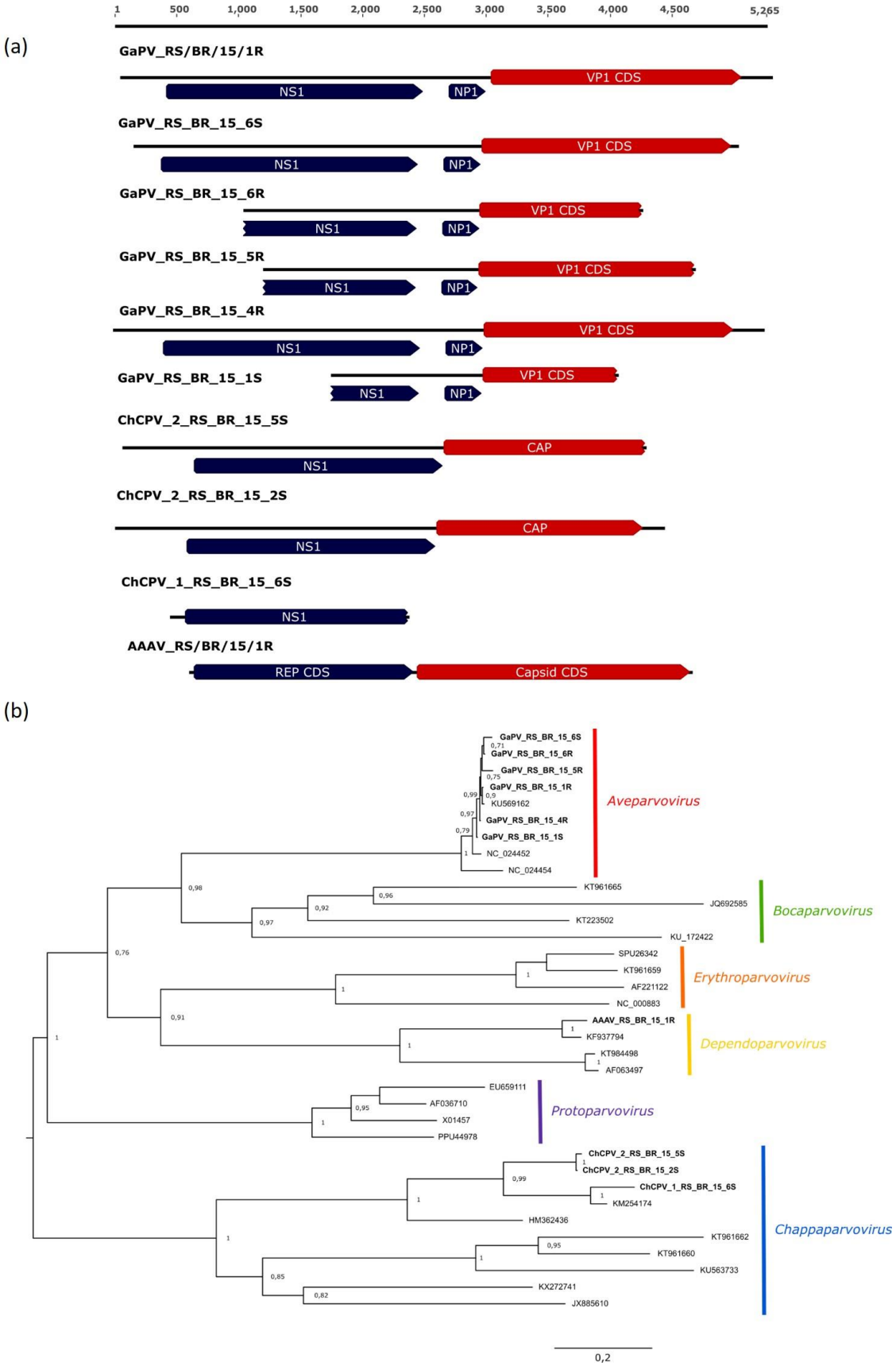
837 Fig. 6



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840 Fig. 7

Virus	Mean difference MAS-affected - Healthy *	P-values (Mann-W
Chicken megrivirus	69828	0.549
Chicken sicinivirus	24401	1.000
Chicken phacovirus	119	0.722
Chicken gallivirus	71	0.777
CRESS-DNA VIRUS	-33977	0.847
Chicken associated cyclovirus	-12348	0.436
Chicken associated cyclovirus 2	487	1.000
Chicken anemia virus	-534	1.000
Avian gyrovirus 2	-1385	0.477
Gyrovirus 4	-6287	0.936
Rotavirus D	2364	1.000
Rotavirus F	-2117	0.722
Rotavirus A	-318	0.477
Avian orthoreovirus	-87	0.336
Chicken picobirnavirus	-4415	0.199
Avian nephritis virus	1872	0.722
Chicken astrovirus	242	0.810
Chicken calicivirus	-1157	0.360
Galliform aveparvovirus	733	0.607
Chicken chapparvovirus 1	3	1.000
Chicken chapparvovirus 2	-192	0.477

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843 Fig. 8

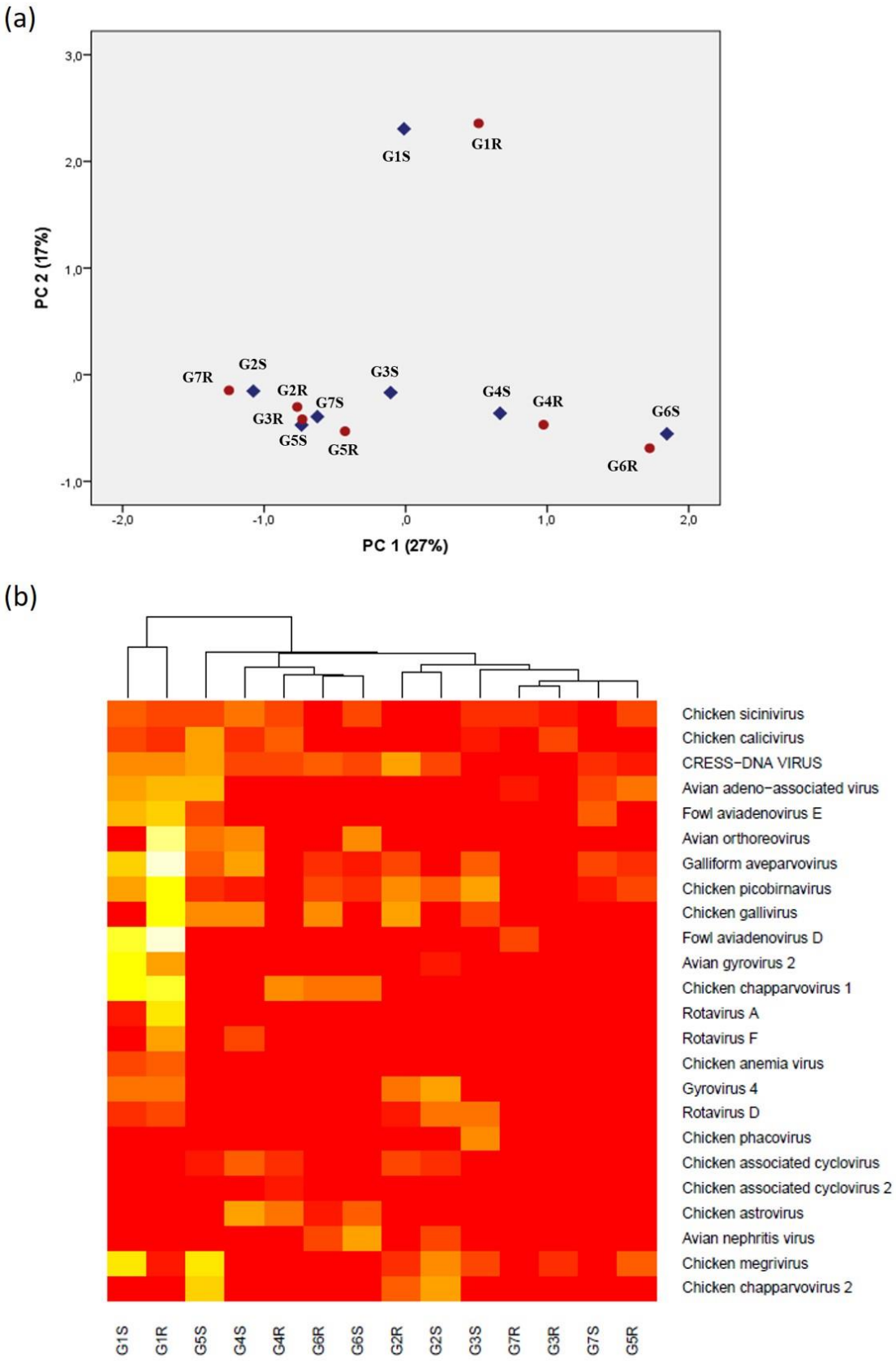

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Table S1. Identification of faecal samples collected from MAS-affected and clinically healthy chickens and total number of reads obtained from each library.

Sample ID	Clinical Condition	Total Reads
G1S	Healthy	540,275
G2S	Healthy	404,658
G3S	Healthy	620,572
G4S	Healthy	388,504
G5S	Healthy	1,124,712
G6S	Healthy	655,242
G7S	Healthy	577,900
G1R	MAS-affected	1,027,030
G2R	MAS-affected	536,460
G3R	MAS-affected	545,040
G4R	MAS-affected	630,826
G5R	MAS-affected	506622
G6R	MAS-affected	544,556
G7R	MAS-affected	244,922

Table S2. Genomes of eukaryotic viruses identified in faecal samples from MAS-affected and healthy and chickens.

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
Chicken megrivirus						
ChMGV_RS_BR_15_2S	1,226	MG846455	AHN10843.1	6E-96	92	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_1R_3	1,282	MG846456	AIV42072.1	0	99	polyprotein [Chicken proventriculitis virus]
ChMGV_RS_BR_15_5S_2	1,468	MG846457	AIV42072.1	0	99	polyprotein [Chicken proventriculitis virus]
ChMGV_RS_BR_15_1R_2	1,542	MG846458	AHN10843.1	8E-167	94	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_5S_1	4,219	MG846459	AHN10843.1	0	94	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_3S_1	4,536	MG846460	YP_009021444.1	0	88	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_1R_1	4,810	MG846461	AHN10843.1	0	96	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_3S_2	5,059	MG846462	AIV42072.1	0	98	polyprotein [Chicken proventriculitis virus]
ChMGV_RS_BR_15_2R	9,555	MG846463	AIV42072.1	0	96	polyprotein [Chicken proventriculitis virus]
ChMGV_RS_BR_15_5R	9,563	MG846464	AHN10843.1	0	96	polyprotein [Chicken megrivirus]
ChMGV_RS_BR_15_3R	9,577	MG846465	AIV42072.1	0	93	polyprotein [Chicken proventriculitis virus]
Chicken sicinivirus						
ChSiV_RS_BR_15_7R_2	1,023	MG846466	AND46625.1	0	94	polyprotein [Sicinivirus 10]
ChSiV_RS_BR_15_1R_3	1,025	MG846467	AIK23440.1	8E-164	77	polyprotein [Chicken picornavirus 1]
ChSiV_RS_BR_15_7R_1	1,065	MG846468	APR73491.1	0	92	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_2R_5	1,073	MG846469	YP_009021767.1	7E-174	87	leader [Sicinivirus A]
ChSiV_RS_BR_15_2S_2	1,131	MG846470	AIK23440.1	0	86	polyprotein [Chicken picornavirus 1]
ChSiV_RS_BR_15_2R_3	1,149	MG846471	APR73490.1	3E-170	85	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_1R_2	1,236	MG846472	AIK23440.1	0	88	polyprotein [Chicken picornavirus 1]
ChSiV_RS_BR_15_2R_4	1,502	MG846473	AIK23440.1	0	82	polyprotein [Chicken picornavirus 1]
ChSiV_RS_BR_15_3S_4	1,548	MG846474	APR73490.1	0	89	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_2R_2	1,696	MG846475	APR73491.1	0	92	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_3S_3	1,723	MG846476	APR73491.1	0	98	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_3S_2	2,587	MG846477	APR73490.1	0	95	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_2R_1	3,832	MG846478	APR73490.1	0	96	polyprotein [Sicinivirus sp.]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
ChSiV_RS_BR_15_3S_1	4,486	MG846479	APR73490.1	0	80	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_2S_1	8,710	MG846480	APR73490.1	0	88	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_4R	9,075	MG846481	APR73490.1	0	89	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_3R	9,240	MG846482	APR73490.1	0	88	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_1R_1	9,270	MG846483	APR73490.1	0	97	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_7S	9,471	MG846484	APR73491.1	0	88	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_4S	9,547	MG846485	APR73491.1	0	94	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_5S	9,711	MG846486	APR73491.	0	94	polyprotein [Sicinivirus sp.]
ChSiV_RS_BR_15_5R	9,874	MG846487	YP_009021568.1	0	92	polyprotein [Sicinivirus A]
Chicken phacovirus						
ChPhV_RS_BR_15_1R_1	4,104	MG846489	ALR74732.1	0	87	polyprotein [Phacovirus Pf-CHK1/PhV]
ChPhV_RS_BR_15_1R_2	3,925	MG846490	ALR74732.1	0	99	polyprotein [Phacovirus Pf-CHK1/PhV]
Chicken gallivirus						
ChGV_RS_BR_15_2R	2,226	MG846488	YP_009054903.1	0	99	polyprotein [Chicken gallivirus 1]
Unclassified CRESS-DNA viruses						
Chicken associated smacovirus RS_BR_15_1R_3	2,343	MG846351	APR73542.1	0	100	capsid protein [Chicken associated smacovirus]
Chicken associated smacovirus RS_BR_15_1R_2	2,408	MG846352	APR73548.1	2,00E-176	100	capsid protein [Chicken associated smacovirus]
Chicken associated smacovirus RS_BR_15_1R_4	2,447	MG846353	APR73546.1	0	93	capsid protein [Chicken associated smacovirus]
Chicken associated smacovirus RS_BR_15_1R_1	2,467	MG846354	YP_009337833.1	0	95	capsid protein [Chicken associated smacovirus]
Chicken stool associated circular virus 2 RS_BR_15_1R	3,161	MG846355	ASU55900.1	7,00E-129	59	replication associated protein [Hudisavirus sp.]
Chicken stool associated circular virus 1 RS_BR_15_1R	3,173	MG846356	ASU55900.1	8,00E-88	63	replication associated protein [Hudisavirus sp.]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
Chicken stool-associated gemycircularvirus RS_BR_15_5S	2,161	MG846357	YP_009337824.1	1,00E-130	100	putative rep protein [Chicken stool-associated gemycircularvirus]
Chicken associated cyclovirus						
ChCyV_2_RS_BR_15_4R	1,778	MG846358	AGJ74756.1	1,00E-82	48	putative rep protein [Dragonfly associated cyclovirus 6]
ChCyV_RS_BR_15_4R	1,902	MG846359	YP_009380542.1	0	98	putative rep protein [Duck associated cyclovirus 1]
ChCyV_RS_BR_15_2S	1,902	MG846360	YP_009380542.1	0	98	putative rep protein [Duck associated cyclovirus 1]
ChCyV_RS_BR_15_4S	1,905	MG846361	YP_009380542.1	0	99	putative rep protein [Duck associated cyclovirus 1]
ChCyV_RS_BR_15_5S	1,905	MG846362	YP_009380542.1	0	99	putative rep protein [Duck associated cyclovirus 1]
Chicken anemia virus						
CAV_RS_BR_15_1R	2,298	MG846491	AOV94162.1	0	99	VP1 [Chicken anemia virus]
Avian gyrovirus 2						
AGV2_RS_BR_15_2S	2,383	MG846492	YP_004376205.1	0	99	putative nucleocapsid [Avian gyrovirus 2]
Gyrovirus 4						
GyV4_RS_BR_15_1R	2,036	MG846493	APQ44722.1	0	99	VP1 [Gyrovirus 4]
GyV4_RS_BR_15_2S	1,539	MG846494	APQ44722.1	0	97	VP1 [Gyrovirus 4]
Rotavirus A						
RVA_RS_BR_15_4S_1	1,016	MG846363	AIW53338.1	0	99	VP2 [Rotavirus A]
Rotavirus D						
RVD_RS_BR_15_2R_5	1,017	MG846364	YP_003896053.1	0	96	NSP2 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_8	1,052	MG846365	YP_003896054.1	0	89	VP7 [Rotavirus D chicken/05V0049/DEU/2005]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
RVD_RS_BR_15_1R_7	1,139	MG846366	AIW53354.1	0	99	VP6 [Rotavirus D]
RVD_RS_BR_15_2R_6	1,267	MG846367	AIW53354.1	0	99	VP6 [Rotavirus D]
RVD_RS_BR_15_1R_6	1,270	MG846368	YP_003896052.1	1,00E-154	94	NSP3 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_2R_7	1,296	MG846369	APR73519.1	0	99	VP1 [Rotavirus D]
RVD_RS_BR_15_2R_4	1,684	MG846370	YP_003896047.1	0	95	VP2 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_2R_3	1,794	MG846371	YP_003896050.1	0	95	NSP1 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_5	1,908	MG846372	YP_003896050.1	0	95	NSP1 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_2R_2	2,048	MG846373	YP_003896048.1	0	93	VP4 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_2R_1	2,062	MG846374	YP_003896049.1	0	88	VP3 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_4	2,098	MG846375	YP_003896049.1	0	89	VP3 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_3	2,321	MG846376	YP_003896048.1	0	93	VP4 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_2	2,789	MG846377	YP_003896047.1	0	96	VP2 [Rotavirus D chicken/05V0049/DEU/2005]
RVD_RS_BR_15_1R_1	3,302	MG846378	YP_003896046.1	0	94	VP1 [Rotavirus D chicken/05V0049/DEU/2005]
Rotavirus F						
RVF_RS_BR_15_5R	1,011	MG846379	YP_008145316.1	0	92	NSP2 [Rotavirus F chicken/03V0568/DEU/2003]
RVF_RS_BR_15_4S_8	1,052	MG846380	AIW53375.1	0	96	VP6 [Rotavirus F]
RVF_RS_BR_15_4S_7	1,079	MG846381	YP_008145316.1	0	94	NSP2 [Rotavirus F chicken/03V0568/DEU/2003]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
RVF_RS_BR_15_4S_6	1,289	MG846382	YP_008145321.1	0	92	NSP3 [Rotavirus F chicken/03V0568/DEU/2003]
RVF_RS_BR_15_4S_5	1,749	MG846383	YP_008145320.1	0	95	NSP1 [Rotavirus F chicken/03V0568/DEU/2003]
RVF_RS_BR_15_4S_4	2,038	MG846384	YP_008145318.1	0	91	VP3 [Rotavirus F chicken/03V0568/DEU/2003]
RVF_RS_BR_15_4S_3	2,128	MG846385	AIW53374.1	0	91	VP4 [Rotavirus F]
RVF_RS_BR_15_4S_2	2,450	MG846386	AIW53371.1	0	96	VP1 [Rotavirus F]
RVF_RS_BR_15_4S_1	2,726	MG846387	YP_008145314.1	0	98	VP2 [Rotavirus F chicken/03V0568/DEU/2003]
Avian orthoreovirus						
ARV_RS_BR_15_6S	1,157	MG846388	AIS22904.1	0	99	lambda-A protein [Avian orthoreovirus]
Chicken picobirnavirus						
ChPBV_RS_BR_15_1S_4	1,016	MG846389	YP_009241385.1	2,00E-117	56	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_4S_2	1,055	MG846390	YP_009241385.1	1,00E-115	54	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_1S_3	1,135	MG846391	AIY31266.1	1,00E-76	47	capsid protein [Dromedary picobirnavirus]
ChPBV_RS_BR_15_5R_2	1,318	MG846392	AMP18944.1	2,00E-89	40	capsid protein [Otarine picobirnavirus]
ChPBV_RS_BR_15_1S_2	1,442	MG846393	AHI59996.1	1,00E-144	47	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_6R_2	1,453	MG846394	AHI59994.1	4,00E-112	46	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_6S_2	1,723	MG846395	AOW41970.1	6,00E-69	32	capsid protein [Picobirnavirus sp.]
ChPBV_RS_BR_15_6R_1	2,095	MG846396	AHI59996.1	3,00E-141	45	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_6S_1	2,118	MG846397	AHI59996.1	8,00E-140	46	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_4S_1	2,406	MG846398	YP_009241385.1	0	53	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_5S_2	2,495	MG846399	YP_009241385.1	0	55	capsid protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_5S_1	2,767	MG846400	AMP18944.1	7,00E-154	46	capsid protein [Otarine picobirnavirus]
ChPBV_RS_BR_15_4S_4	1,007	MG846401	AIW53311.1	0	89	RdRp protein [Picobirnavirus HK-2014]
ChPBV_RS_BR_15_4S_3	1,009	MG846402	ASM93465.1	1,00E-178	74	RdRp protein [Porcine picobirnavirus]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
ChPBV_RS_BR_15_5S_6	1,021	MG846403	YP_009389484.1	0	79	RdRp protein [Picobirnavirus dog/KNA/2015]
ChPBV_RS_BR_15_6R_5	1,070	MG846404	ANS53885.1	2,00E-172	69	RdRp protein [Picobirnavirus wolf/PRT/416/2015]
ChPBV_RS_BR_15_6R_4	1,121	MG846405	AGK45545.1	5,00E-161	61	RdRp protein [Fox picobirnavirus]
ChPBV_RS_BR_15_6S_3	1,258	MG846406	BAJ53294.1	0	69	RdRp protein [Human picobirnavirus]
ChPBV_RS_BR_15_5S_5	1,260	MG846407	YP_009241386.1	0	74	RdRp protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_5R_1	1,336	MG846408	ANS53885.1	0	71	RdRp protein [Picobirnavirus wolf/PRT/416/2015]
ChPBV_RS_BR_15_5S_4	1,400	MG846409	YP_009241386.1	0	80	RdRp protein [Porcine picobirnavirus]
ChPBV_RS_BR_15_6R_3	1,418	MG846410	BAJ53294.1	0	68	RdRp protein [Human picobirnavirus]
ChPBV_RS_BR_15_5S_3	1,607	MG846411	ANS53886.1	0	69	RdRp protein [Picobirnavirus wolf/PRT/1109/2015]
ChPBV_RS_BR_15_1S_1	1,674	MG846412	YP_009241386.1	0	84	RdRp protein [Porcine picobirnavirus]
Avian nephritis virus						
ANV_RS_BR_15_6S_1	1,162	MG846413	AJM71334.1	0	88	non-structural polyprotein [Avian nephritis virus]
ANV_RS_BR_15_6S_2	1,949	MG846414	AOR81688.1	0	98	capsid protein [Avian nephritis virus]
ANV_RS_BR_15_6R	6,890	MG846415	AEQ33643.1	0	78	non-structural polyprotein [Pigeon avian nephritis virus]
Chicken astrovirus						
CAstV_RS_BR_15_6R_3	1,425	MG846416	AFK92946.1	0	91	capsid protein [Chicken astrovirus]
CAstV_RS_BR_15_6S	1,480	MG846417	AIW53316.1	0	96	non-structural polyprotein [Astrovirus HK-2014]
CAstV_RS_BR_15_4R_1	2,367	MG846418	AFK92941.1	0	96	capsid protein [Chicken astrovirus]
CAstV_RS_BR_15_4R_2	2,477	MG846419	AEE88303.1	0	97	non-structural polyprotein [Chicken astrovirus]
CAstV_RS_BR_15_6R_2	2,512	MG846420	NP_059947.1	0	96	orf1a polyprotein (pp1a) [Turkey astrovirus]
CAstV_RS_BR_15_6R_1	3,204	MG846421	AOR81715.1	0	95	capsid protein [Turkey astrovirus 1]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
Chicken calicivirus						
ChCaV_RS_BR_15_5S_2	1,078	MG846422	YP_009337240.1	1E-135	91	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_4R_2	1,196	MG846423	ADN88287.1	0	99	polyprotein [Calicivirus chicken/V0021/Bayern/2004]
ChCaV_RS_BR_15_5S_1	1,327	MG846424	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_6S_2	1,605	MG846425	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_4R_3	1,880	MG846426	AIW53323.1	0	93	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_6S_1	2,263	MG846427	AIW53323.1	0	94	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_4R_1	2,540	MG846428	YP_009337240.1	0	96	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_3S_1	3,045	MG846429	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_4S_1	3,155	MG846430	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_3S_2	3,783	MG846431	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_4S_2	4,879	MG846432	YP_009337240.1	0	98	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_6R	8,187	MG846433	YP_009337240.1	0	97	polyprotein [Chicken calicivirus]
ChCaV_RS_BR_15_1R_1	8,374	MG846434	YP_009337240.1	0	99	polyprotein [Chicken calicivirus]
Galliform aveparvovirus						
GaPV_RS_BR_15_1S	2,318	MG846435	AIW53327.1	0	99	VP1 [Protoparvovirus HK-2014]
GaPV_RS_BR_15_6R	3,221	MG846436	AMZ04136.1	0	99	nonstructural protein [Galliform aveparvovirus 1]
GaPV_RS_BR_15_5R	3,492	MG846437	AMZ04138.1	0	97	VP1 [Galliform aveparvovirus 1]
GaPV_RS_BR_15_6S	4,886	MG846438	AIW53325.1	0	99	NS1 [Protoparvovirus HK-2014]
GaPV_RS_BR_15_4R	5,256	MG846439	AMZ04136.1	0	100	nonstructural protein [Galliform aveparvovirus 1]
GaPV_RS_BR_15_1R	5,265	MG846440	AMZ04136.1	0	99	nonstructural protein [Galliform aveparvovirus 1]
Chicken chapparvovirus 1						
ChCPV_1_RS_BR_15_6S	1,930	MG846441	AIW53333.1	0	99	NS1 [Protoparvovirus HK-2014]

Table S2 continued

Virus	Size	GenBank	GenBank Reference	E-value	Identity BLASTx (%)	Closest relative
Chicken chapparvovirus 2						
ChCPV_2_RS_BR_15_2S	4,432	MG846442	AIW53333.1	0	75	NS1 [Protoparvovirus HK-2014]
ChCPV_2_RS_BR_15_5S	4,228	MG846443	AIW53333.1	0	74	NS1 [Protoparvovirus HK-2014]
Avian adeno-associated virus						
AAAV_RS_BR_15_1R	4,062	MG846444	ACU30842.1	0	94	VP1 [Avian adeno-associated virus]
Fowl aviadenovirus D						
FAvD_RS_BR_15_1R_10	1,017	MG846445	APQ44775.1	5E-82	100	ORF23 [Fowl adenovirus]
FAvD_RS_BR_15_1R_9	1,036	MG846446	NP_050280.1	0	99	maturation protein [Fowl aviadenovirus D]
FAvD_RS_BR_15_1R_8	1,109	MG846447	NP_050286.1	4E-100	99	minor capsid protein precursor [Fowl aviadenovirus D]
FAvD_RS_BR_15_1R_7	1,260	MG846448	ANJ02618.1	3E-89	93	ORF11 [Fowl aviadenovirus 11]
FAvD_RS_BR_15_1R_6	1,261	MG846449	APQ44773.1	3E-141	99	triacylglycerol lipase-like protein [Fowl aviadenovirus D]
FAvD_RS_BR_15_1R_5	1,401	MG846450	ABB18324.1	0	99	ORF2 [Fowl aviadenovirus 2]
FAvD_RS_BR_15_1R_4	1,555	MG846451	AKR76208.1	0	99	ORF19 protein [Fowl aviadenovirus D]
FAvD_RS_BR_15_1R_3	2,036	MG846452	ANJ02347.1	0	99	hexon assembly protein 100K [Fowl aviadenovirus 2]
FAvD_RS_BR_15_1R_2	2,378	MG846453	NP_597825.1	0	96	hypothetical protein FAdVDgp23 [Fowl aviadenovirus D]
FAvD_RS_BR_15_1R_1	2,422	MG846454	NP_050281.1	0	98	DNA polymerase [Fowl aviadenovirus D]

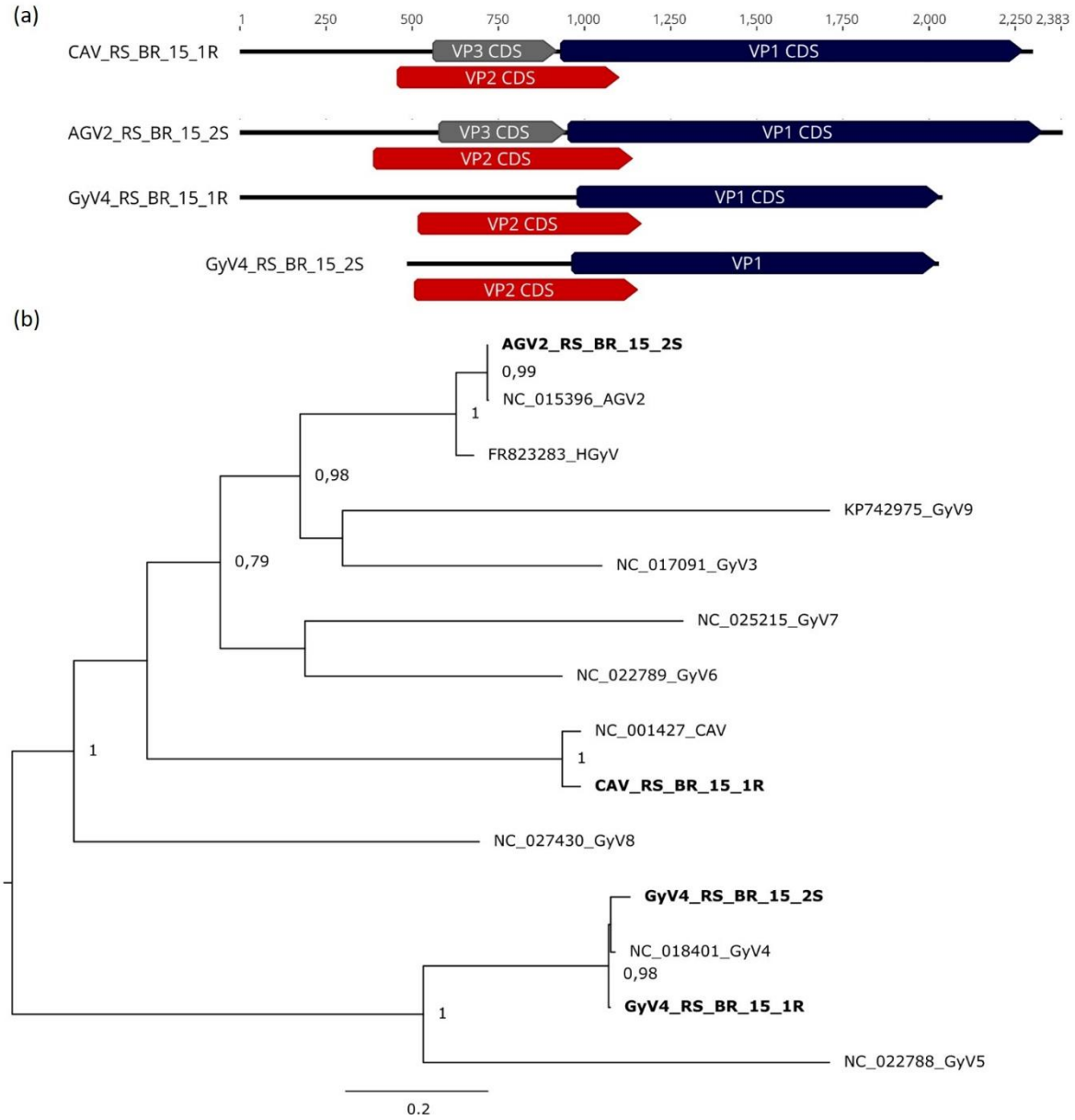

Fig. S1. Schematic representation and phylogenetic analysis of gyrovirus genomes. (a) Genomic organization of CAV, AGV2 and GyV4 describe in this study. (b) Maximum likelihood phylogenetic tree based on VP1 nucleotide sequences with GTR substitution model. The branch support is evidenced only for the clades with an aLRT greater than 70 %. The gyroviruses described here are shown in bold.

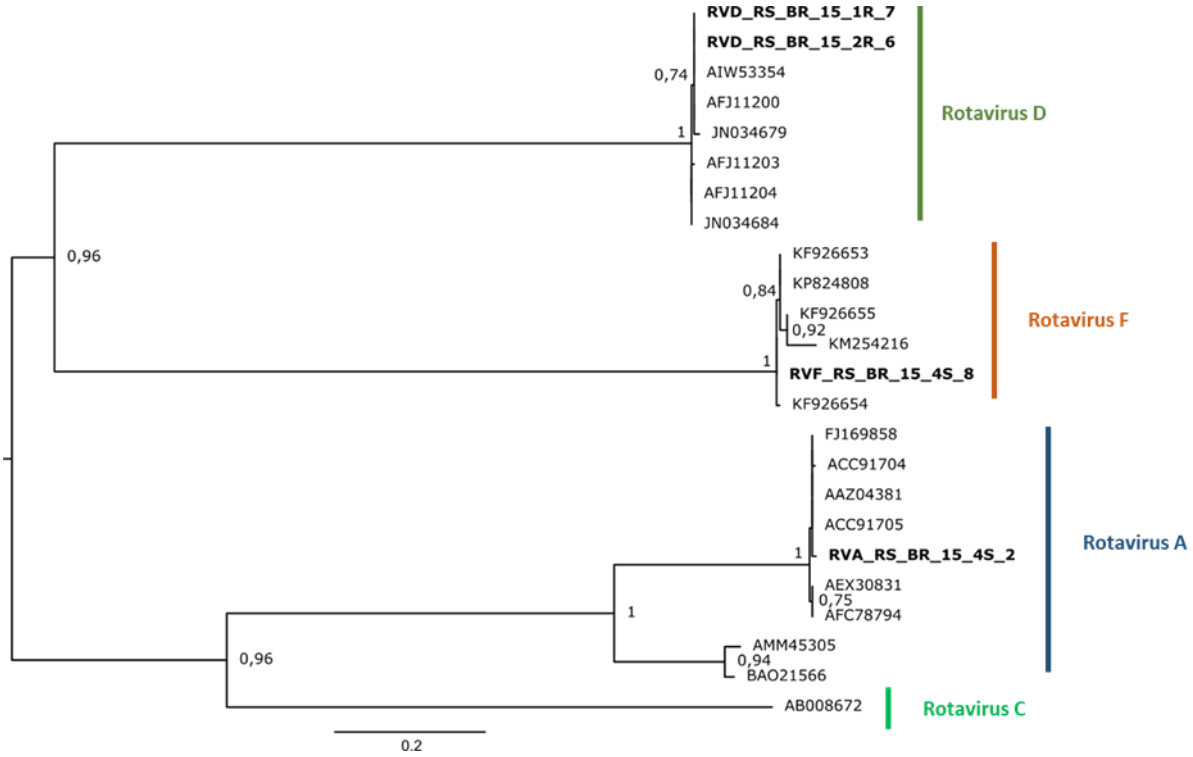

Fig. S2. Maximum likelihood phylogenetic tree reconstructed with the substitution model GTR of VP2 nucleotide sequences from representative members of rotavirus A, D and F species. Branch support is evidenced only for the clades with an aLRT support greater than 70 % .The rotavirus contigs recovered in this study are shown in bold.

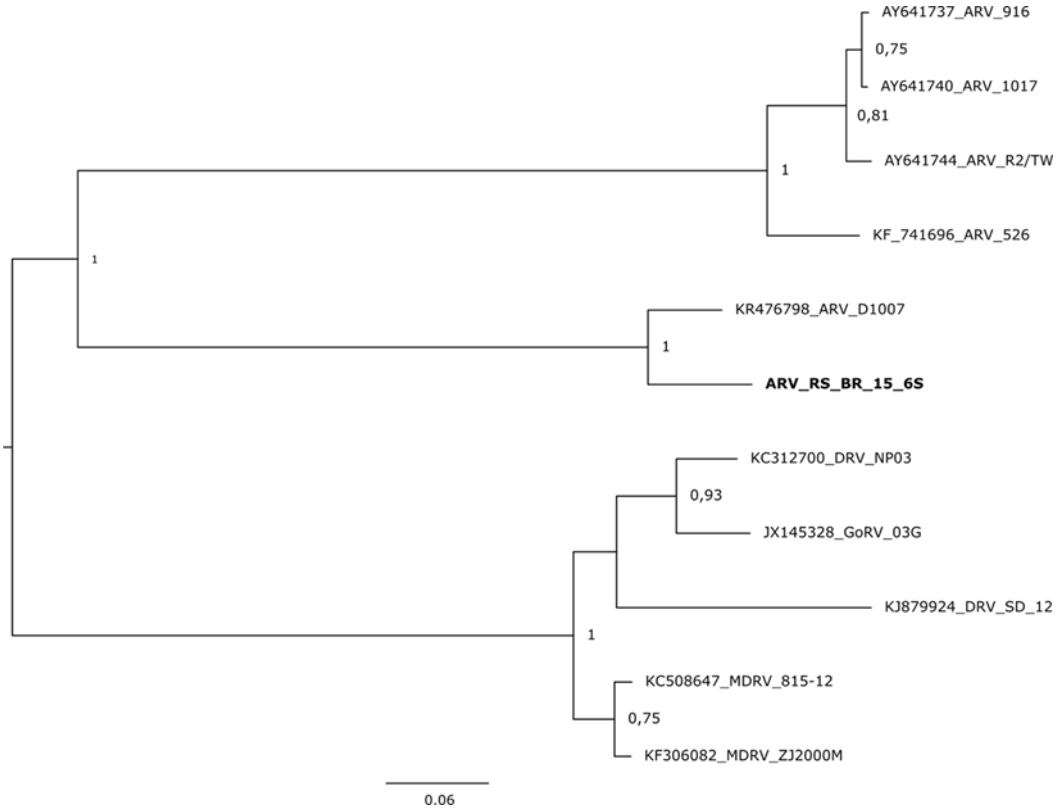

Fig. S3. Maximum likelihood phylogenetic tree of the lambda-A nucleotide sequence of avian orthoreoviruses, constructed with the best fit substitution model GTR+I. Branch support is evidenced only for the clades with an aLRT support greater than 70%. The avian orthoreovirus contig recovered here is shown in bold.

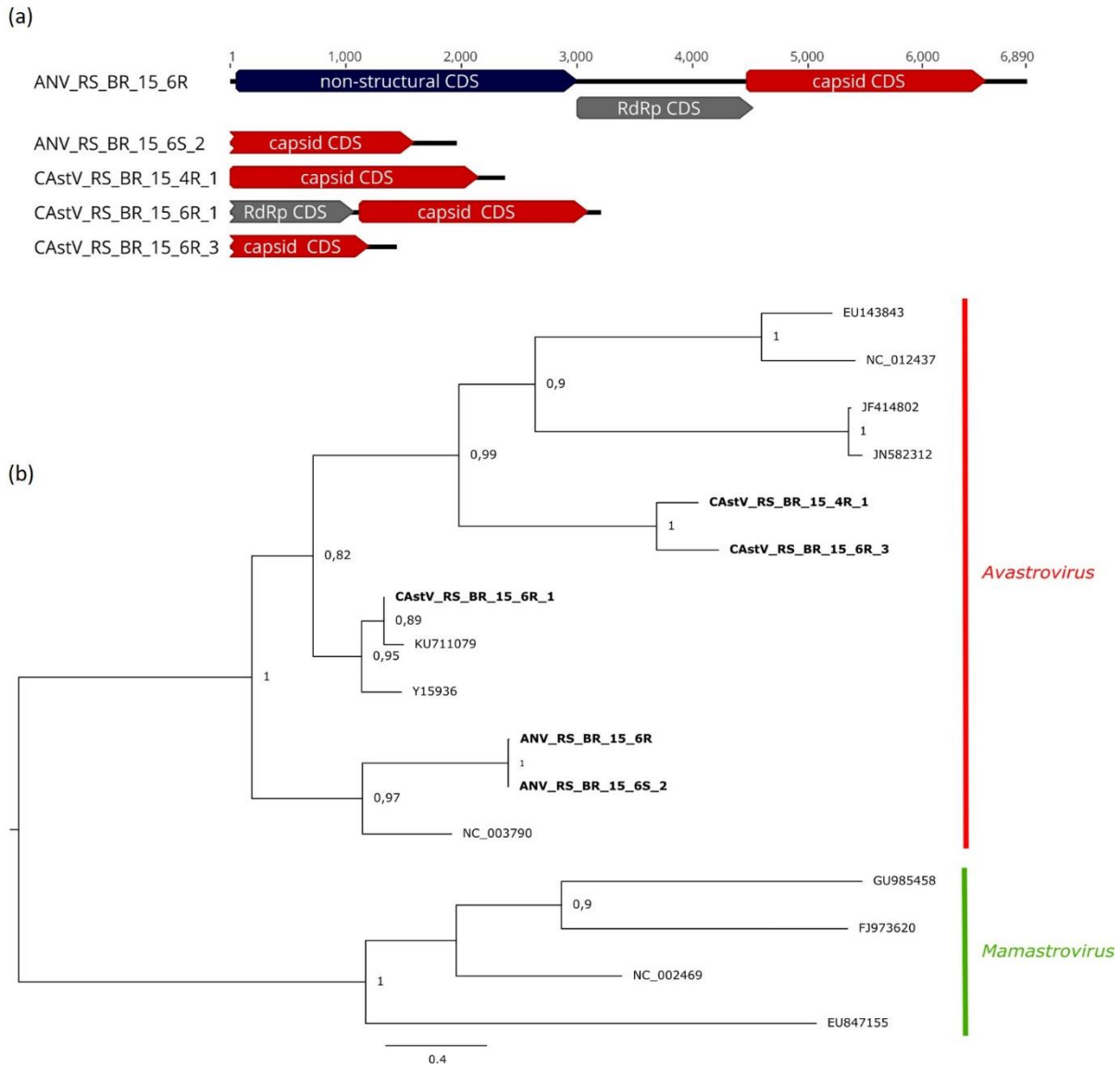

Fig. S4. Schematic representation and phylogenetic analysis of astrovirus genomes. (a) Putative ORF maps of avian nephritis virus and chicken astrovirus contigs recovered from feces of MAS-affected and healthy chickens. (b) Maximum likelihood phylogenetic tree reconstructed with the substitution model LG+I+G+F of capsid amino acid sequences from representative members of *Avastrovirus* and *Mamastrovirus* genera. Branch support is evidenced only for the clades with an aLRT support greater than 70 %.The astroviruses recovered in this study are shown in bold.

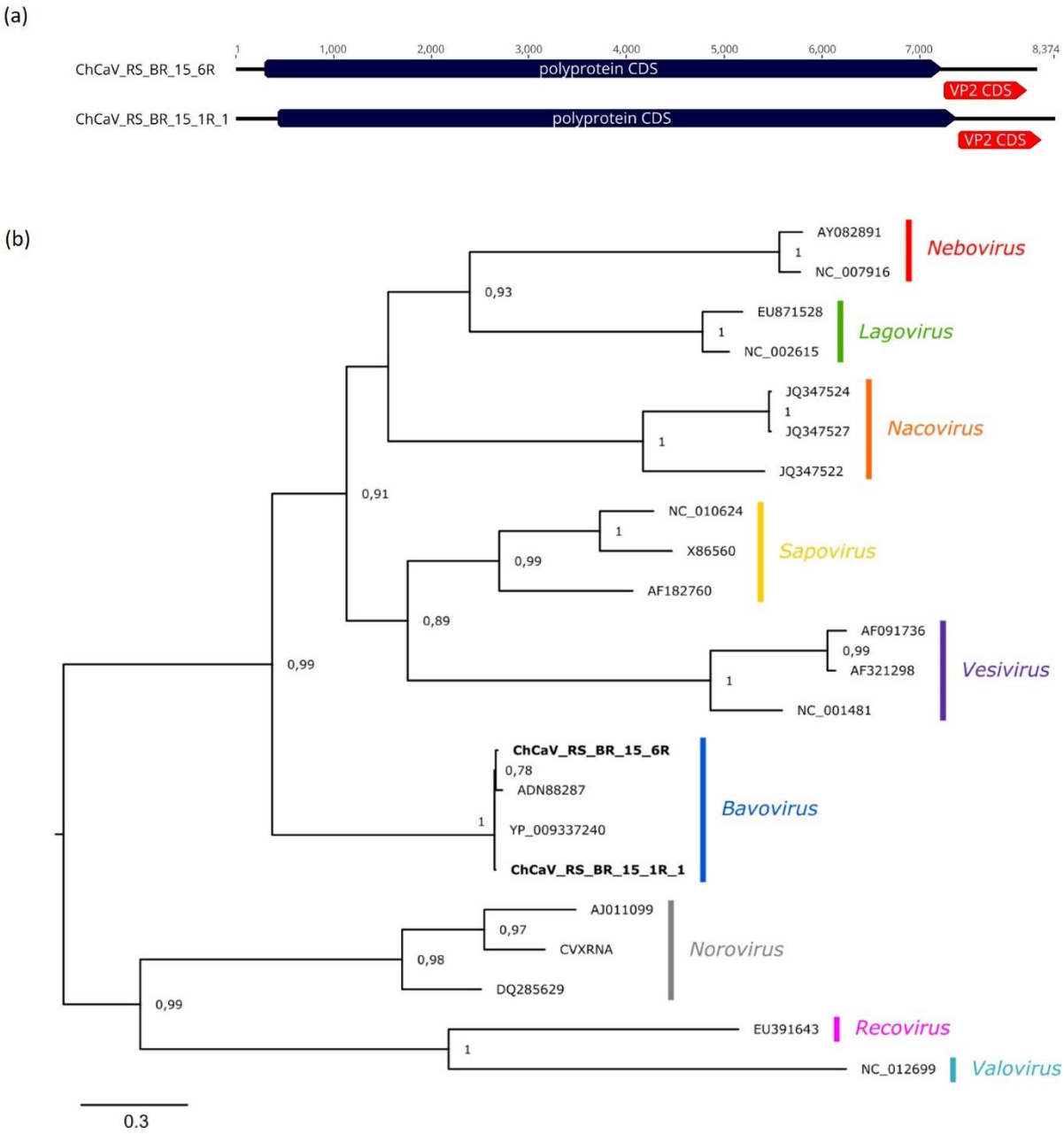

Fig. S5. Putative genome organization and phylogenetic analysis of chicken caliciviruses. (a) Schematic representation of the caliciviruses contigs recovered from chicken feces in this study. (b) Maximum likelihood phylogenetic tree reconstructed with the substitution model RtREV+G+I+F of RdRp amino acid sequences from representative members of *Caliciviridae* family. The branch support is evidenced only for the clades with an aLRT support greater than 70 %. The chicken caliciviruses reported here are shown in bold.

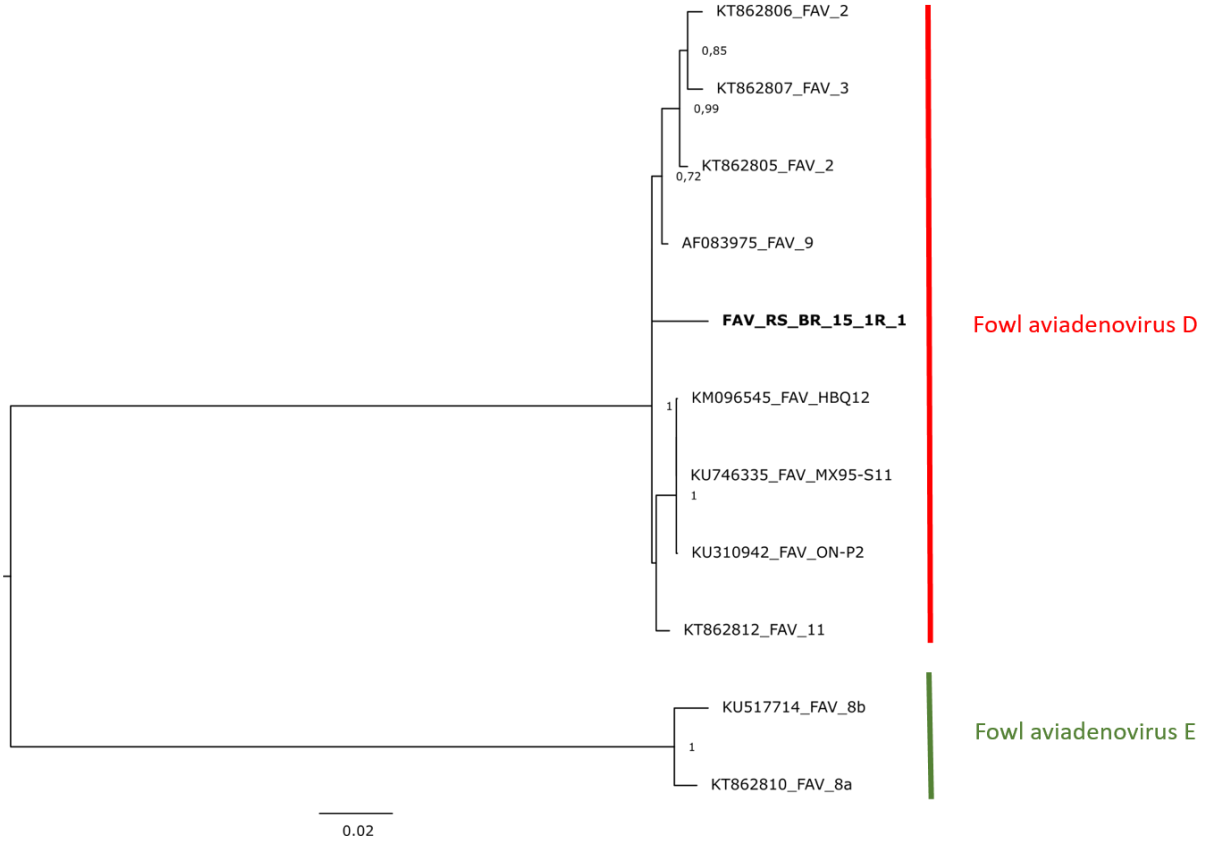

Fig. S6. Maximum likelihood phylogenetic tree reconstructed with the substitution model GTR+G+I on DNA polymerase nucleotide sequences from representative members of fowl aviadenovirus species D and E. The branch support is evidenced only for the clades with an aLRT greater than 70 %. The fowl aviadenovirus D sequence reported in this study is shown in bold.

6 CONCLUSÕES:

- A utilização da metagenômica para caracterização do viroma intestinal de frangos de corte doentes e saudáveis revelou ampla variedade de genomas de vírus DNA e RNA, representantes de diversas famílias virais.

- Foram identificados vários genomas de agentes previamente desconhecidos e constituídos de genoma DNA circular fita simples, descritos como vírus CRESS-DNA.

- Foram detectadas novas espécies virais pertencentes aos gêneros *Cyclovirus* (família Circoviridae), *Chapparvovirus* (*Parvoviridae*) e *Picobirnavirus* (*Picobirnaviridae*).

- A distribuição de sequências virais correspondentes aos vírus eucarióticos identificados neste estudo não apresentou diferença estatística quando comparados os grupos de aves afetadas pela SMA e de aves saudáveis.

- Da mesma forma, não houve distinção entre o perfil dos viromas identificados nas aves acometidas pela SMA e aqueles identificados nas aves clinicamente saudáveis. Estes resultados sustentam a hipótese de que não há um agente viral específico associado à ocorrência de MAS.

REFERÊNCIAS

ABPA, A. B. P. A. Relatório Anual ABPA 2016. 2017. Disponível em: <http://abpa-br.com.br/storage/files/3678c_final_abpa_relatorio_anual_2016_portugues_web_reduzido.pdf>. Acesso em: 06 fev 2017.

ALFIERI, A. A. et al. Reoviridae. In: FLORES, E. (Ed.). **Virologia Veterinária**. Santa Maria: Editora UFSM, 2007. p.773-807.

ALFIERI, A. F.; TAMEHIRO, C. Y.; ALFIERI, A. A. Vírus entéricos RNA fita dupla, segmentado, em aves: rotavírus, reovírus e picobirnavírus. **Semina: Ci. Agrárias,** v. 21, n. 1, p. 101-113, 2000.

ALHAMLAN, F. S. et al. Metagenomics-based analysis of viral communities in dairy lagoon wastewater. **J Microbiol Methods,** v. 92, n. 2, p. 183-8, Feb 15 2013.

ANDREATTI FILHO, R. L. Prevenção de Doenças: Biosseguridade em Avicultura. . In: ANDREATTI FILHO, R. L. (Ed.). **Saúde Aviária e Doenças**. São Paulo: Roca, 2007.

ANDREWS, S. FastQC A Quality Control Tool for High Throughput Sequence Data. 2018. Disponível em: < https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> Acesso em: 07 fev 2018.

ANSORGE, W. J. Next-generation DNA sequencing techniques. **New Biotechnology,** v. 25, p. 195-203, 2009.

ATTOUI, H. et al. Family Reoviridae. In: KING, A. M. Q.;ADAMS, M. J., *et al* (Ed.). **Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses**: Elsevier, 2012. cap. Family Reoviridae, p.541-554.

AWANDKAR, S. P. et al. Comparative investigations of infectious runting and stunting syndrome in vaccinated breeder chicks by inactivated reovirus and chicks from non-vaccinated breeders. **Iran J Vet Res,** v. 18, n. 1, p. 6-12, 2017.

AYALEW, L. E. et al. Phenotypic, genotypic and antigenic characterization of emerging avian reoviruses isolated from clinical cases of arthritis in broilers in Saskatchewan, Canada. **Sci Rep,** v. 7, n. 1, p. 3565, 2017.

BARNES, H. J.; GUY, J. S.; VAILLANCOURT, J. P. Poult enteritis complex. **Rev Sci Tech,** v. 19, n. 2, p. 565-88, 2000.

BARZON, L. et al. Applications of next-generation sequencing technologies to diagnostic virology. **Int J Mol Sci,** v. 12, n. 11, p. 7861-84, 2011.

BAXENDALE, W.; MEBATSION, T. The isolation and characterisation of astroviruses from chickens. **Avian Pathol,** v. 33, n. 3, p. 364-70, 2004.

BELAK, S. et al. New viruses in veterinary medicine, detected by metagenomic approaches. **Vet Microbiol,** v. 165, n. 1-2, p. 95-101, 2013.

BENAVENTE, J.; MARTINEZ-COSTAS, J. Avian reovirus: structure and biology. **Virus Res,** v. 123, n. 2, p. 105-19, 2007.

BERNS, K.; PARRISH, C. R. Parvoviridae. In: KNIPE, D. M. e HOWLEY, P. M. (Ed.). **Fields Virology**. Sixth. Philadelphia: Lippincott Williams & Wilkins, v.2, 2013. cap. 57, p.2688.

BESERRA, L. A. et al. Occurrence and characterization of rotavirus A in broilers, layers, and broiler breeders from Brazilian poultry farms. **Avian Dis,** v. 58, n. 1, p. 153-7, 2014.

BESERRA, L. A.; GREGORI, F. Description of rotavirus F in broilers from Brazilian poultry farms. **Avian Dis,** v. 58, n. 3, p. 458-61, 2014.

BIDIN, M. et al. Identification and phylogenetic diversity of parvovirus circulating in commercial chicken and turkey flocks in Croatia. **Avian Dis,** v. 55, n. 4, p. 693-6, 2011.

BISHOP, R. Discovery of rotavirus: Implications for child health. **J Gastroenterol Hepatol,** v. 24 Suppl 3, p. S81-5, 2009.

BLOMSTROM, A. L. et al. Detection of a novel astrovirus in brain tissue of mink suffering from shaking mink syndrome by use of viral metagenomics. **J Clin Microbiol,** v. 48, n. 12, p. 4392-6, 2010.

BOSCH, A. et al. Family Astroviridae. In: KING, A. M. Q.;ADAMS, M. J., *et al* (Ed.). **Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses**. Birmingham: Academic Press Elsevier, 2012. p.953-959.

BREITBART, M. et al. Diversity and population structure of a near-shore marine-sediment viral community. **Proc Biol Sci,** v. 271, n. 1539, p. 565-74, 2004.

BREITBART, M.; ROHWER, F. Here a virus, there a virus, everywhere the same virus? . **Trends in Microbiology,** v. 13, p. 278-284, 2005.

BZHALAVA, D.; DILLNER, J. Bioinformatics for viral metagenomics. **J Data Mining Genomics Proteomics,** v. 271, p. 565-574, 2013.

CANELLI, E. et al. Astroviruses as causative agents of poultry enteritis: genetic characterization and longitudinal studies on field conditions. **Avian Dis,** v. 56, n. 1, p. 173-82, 2012.

CARDING, S. R.; DAVIS, N.; HOYLES, L. Review article: the human intestinal virome in health and disease. **Aliment Pharmacol Ther,** v. 46, n. 9, p. 800-815, 2017.

CATTOLI, G. et al. Astroviruses found in the intestinal contents of guinea fowl suffering from enteritis. . **Veterinary Record,** v. 156, n. 220, 2005.

CHEN, F. et al. Genome characterization of Turkey Rotavirus G strains from the United States identifies potential recombination events with human Rotavirus B strains. **J Gen Virol,** v. 98, n. 12, p. 2931-2936, 2017.

CHEN, G. Q. et al. Identification and survey of a novel avian coronavirus in ducks. **PLoS One,** v. 8, n. 8, p. e72918, 2013.

CINGOLANI, P.; SLADEK, R.; BLANCHETTE, M. BigDataScript: a scripting language for data pipelines. **Bioinformatics,** v. 31, n. 1, p. 10-6, 2015.

CORREA-FIZ, F.; FRAILE, L.; ARAGON, V. Piglet nasal microbiota at weaning may influence the development of Glasser's disease during the rearing period. **BMC Genomics,** v. 17, p. 404, 2016.

COTMORE, S. F. et al. The family Parvoviridae. **Arch Virol,** v. 159, n. 5, p. 1239-47, 2014.

DAY, J. M. The diversity of the orthoreoviruses: molecular taxonomy and phylogentic divides. **Infect Genet Evol,** v. 9, n. 4, p. 390-400, 2009.

DAY, J. M. et al. Metagenomic analysis of the turkey gut RNA virus community. **Virol J,** v. 7, p. 313, 2010.

DAY, J. M. et al. Comparative analysis of the intestinal bacterial and RNA viral communities from sentinel birds placed on selected broiler chicken farms. **PLoS One,** v. 10, n. 1, 2015.

DAY, J. M.; SPACKMAN, E.; PANTIN-JACKWOOD, M. J. A Multiplex RT-PCR test for the diferencial identification of turkey astrovirus type 1, turkey astrovirus type 2,

chicken astrovirus, avian nephritis virus, and avian rotavirus. . **Avian Diseases,** v. 51, p. 681-684, 2007.

DAY, J. M.; ZSAK, L. Determination and analysis of the full-length chicken parvovirus genome. **Virology,** v. 399, n. 1, p. 59-64, 2010.

DAY, J.M; ZSAK, L. Recent progress in the characterization of avian enteric viruses. **Avian Dis,** v. 57, n. 3, p. 573-80, 2013.

DAYARAM, A. et al. Diverse circular replication-associated protein encoding viruses circulating in invertebrates within a lake ecosystem. **Infect Genet Evol,** v. 39, p. 304-316, 2016.

DAYARAM, A. et al. Diverse small circular DNA viruses circulating amongst estuarine molluscs. **Infect Genet Evol,** v. 31, p. 284-95, 2015.

DELWART, E. L. Viral metagenomics. **Rev Med Virol,** v. 17, n. 2, p. 115-31, 2007.

DEOL, P. et al. Avian group D rotaviruses: structure, epidemiology, diagnosis, and perspectives on future research challenges. **Pathogens,** v. 6, n. 4, 2017.

DERMODY, T. S.; PARKER, J. S. L.; SHERRY, B. Orthoreviruses. In: KNIPE, D. M. e HOWLEY, P. M. (Ed.). **Fields Virology**. 6th. Philadelphia: Lippincott Williams and Wilkins, 2013. p.1304-1346.

DEVANEY, R. et al. A metagenomic comparison of endemic viruses from broiler chickens with runting-stunting syndrome and from normal birds. **Avian Pathol,** v. 45, n. 6, p. 616-629, 2016.

DI BELLA, J. M. et al. High throughput sequencing methods and analysis for microbiome research. **Journal of Microbiological Methods,** v. 95, p. 401-414, 2013.

DUDHAGARA, P. et al. Web resources for metagenomics studies. **Genomics Proteomics Bioinformatics,** v. 13, n. 5, p. 296-303, 2015.

EDWARDS, R.; ROHWER, F. Viral metagenomics. **Nature Review Microbiology,** v. 3, p. 504-510, 2005.

ESCOBAR-ZEPEDA, A.; VERA-PONCE DE LEON, A.; SANCHEZ-FLORES, A. The road to metagenomics: from microbiology to dna sequencing technologies and bioinformatics. **Front Genet,** v. 6, p. 348, 2015.

ESTES, M. K.; GREENBERG, H. B. Rotaviruses In: KNIPE, D. M. e HOWLEY, P. M. (Ed.). **Fields Virology**. 6th. Philadelphia: Lippincott Williams and Wilkins, 2013. p.1347-1401.

EWING, B.; GREEN, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. **Genome Res,** v. 8, n. 3, p. 186-94, 1998.

FARKAS, S. L. et al. Lineage diversification, homo- and heterologous reassortment and recombination shape the evolution of chicken orthoreoviruses. **Sci Rep,** v. 6, p. 36960, 2016.

FINKLER, F. et al. Chicken parvovirus viral loads in cloacal swabs from malabsorption syndrome-affected and healthy broilers. **Trop Anim Health Prod,** v. 48, n. 8, p. 1685-1689, 2016.

FINKLER, F. et al. Chicken parvovirus and its associations with malabsorption syndrome. **Res Vet Sci,** v. 107, p. 178-81, 2016.

FISCHER, T. K. et al. Hospitalizations and deaths from diarrhea and rotavirus among children _5 years of age in the United States. **J. Infect. Dis,** v. 195, p. 1117–1125, 2007.

FOSTER, J. A. et al. Measuring the microbiome: perspectives on advances in DNA-based techniques for exploring microbial life. **Brief Bioinform,** v. 13, n. 4, p. 420-9, 2012.

FRAZIER, J. A.; REECE, R. L. Infectious stunting syndrome of chickens in Great Britain: Intestinal ultrastuctural pathology. **Avian Pathology,** v. 19, p. 759-777, 1990.

GRENINGER, A. L. A decade of RNA virus metagenomics is (not) enough. **Virus Res,** v. 244, p. 218-229, 2017.

GUY, J. S. Virus infection of the gastrointestinal tract of poultry. **Poultry Science,** v. 77, p. 1166-1175, 1998.

HANDELSMAN, J. et al. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. **Chem Biol,** v. 5, n. 10, p. R245-9, 1998.

HAUCK, R. et al. A coronavirus associated with runting stunting syndrome in broiler chickens. **Avian Dis,** v. 60, n. 2, p. 528-34, 2016.

HEGGEN-PEAY, C. L. et al. Isolation of a reovirus from poult enteritis and mortality syndrome and its pathogenicity in turkey poults. **Avian Dis,** v. 46, n. 1, p. 32-47, 2002.

ITO NMK, M. C., OKABAYASHI SM. Saúde intestinal em frangos de corte. **Aviagen Brasil Circular Técnica**, 2007. Disponível em: <http://www.aviagen.com/assets/Tech_Center/BB_Foreign_Language_Docs/Portuguese>.

ITO, N. M. K.; MIYAJI, C. I.; OKABAYASHI, S. M. Saúde intestinal em frangos de corte. **Aviagen Brasil Circular Técnica**, 2007. Disponível em: <http://www.aviagen.com/assets/Tech_Center/BB_Foreign_Language_Docs/Portuguese/novembro2007-saudeintestinalemfrangosdecorte.pdf>. Acesso em: 06 fev.

ITO, N. M. K. et al. Fisiologia do sistema digestório e anexos. In: BERCHIERI JÚNIOR, A.;SILVA, E. N., *et al* (Ed.). **Doenças das aves**. 2nd. Campinas: Fundação Apinco de Ciência e Tecnologia Avícolas, 2009. p.215-264.

JINDAL, N. et al. A multiplex RT-PCR for the detection of astrovirus, rotavirus, and reovirus in turkeys. **Avian Dis,** v. 56, n. 3, p. 592-6, 2012.

JINDAL, N.; MOR, S. K.; GOYAL, S. M. Enteric viruses in turkey enteritis. **Virusdisease,** v. 25, n. 2, p. 173-85, 2014.

JINDAL, N. et al. Duration of growth depression and pathogen shedding in experimentally reproduced poult enteritis syndrome. **Avian Dis,** v. 53, n. 4, p. 517-22, 2009.

JOHNE, R. et al. Sequence analysis of the VP6-encoding genome segment of avian group F and G rotaviruses. **Virology,** v. 412, n. 2, p. 384-91, 2011.

JOHNE, R. et al. Generation of an avian-mammalian rotavirus reassortant by using a helper virus-dependent reverse genetics system. **J Virol,** v. 90, n. 3, p. 1439-43, 2015.

JONES, R. C. Reovirus Infections. In: SAIF, Y. M. (Ed.). **Disease of Poultry**. Iowa: Blackwell Publishing, 2008. p.309-310.

KAITHAL, B. et al. Detection and molecular characterization of enteric viruses in enteritis-affected commercial broiler chickens in India. **Acta Virol,** v. 60, n. 4, p. 361-371, 2016.

KANG, K. I. et al. Investigation into the aetiology of runting and stunting syndrome in chickens. **Avian Pathol,** v. 41, n. 1, p. 41-50, 2012.

KISARY, J. Experimental infection of chicken embryos and day-old chickens with parvovirus of chicken origin. . **Avian Pathology,** v. 14, p. 1-7, 1985.

KISARY, J.; NAGY, B.; BITAY, Z. Presence of parvoviruses in the intestine of chickens showing stunting syndrome. . **Avian Pathology,** v. 13, p. 339-343, 1984.

KOCI, M. D.; SCHULTZ-CHERRY, S. Avian astroviruses. **Avian Pathol,** v. 31, n. 3, p. 213-27, 2002.

KOUWENHOVEN, B.; DAVELAAR, F. G.; VAN WALSUN, J. Infectious proventriculitis causing runting in broilers. **Avian Pathology,** v. 7, p. 183-187, 1978.

KOUWENHOVEN, B.; VERTOMMEN, M.; GOREN, E. Investigations into the role of reovirus in the malabsorption syndrome. **Avian Pathology,** v. 17, p. 879-892, 1988.

LANE, D. J. et al. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. **Proc Natl Acad Sci U S A,** v. 82, n. 20, p. 6955-9, 1985.

LAW, J. et al. Identification of hepatotropic viruses from plasma using deep sequencing: a next generation diagnostic tool. **PLoS One,** v. 8, n. 4, p. e60595, 2013.

LIMA, D. A. et al. Faecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses. **J Gen Virol,** v. 98, n. 4, p. 690-703, 2017.

MARTHALER, D. et al. Widespread rotavirus H in commercially raised pigs, United States. **Emerg Infect Dis,** v. 20, n. 7, p. 1195-8, 2014.

MARTINS, A. M. **Sequenciamento de DNA, montagem de novo do genoma e desenvolvimento de marcadores microssatélites, indels e SNPs para uso em análise genética de Brachiaria ruziziensis.** 2013. 198 (Tese de Doutorado). Departamento de Biologia Celular, Universidade de Brasília.

MARTINS, N. R. S.; RESENDE, J. S. Adenoviroses, reoviroses, rotaviruses e viroses intestinais. In: BERCHIERI JÚNIOR, A.;SILVA, E. N., *et al* (Ed.). **Doenças das aves**. Campinas: Fundação Apinco de Ciência e Tecnologia Avícolas, 2009. p.677-710.

MATTHIJNSSENS, J. et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). **Arch Virol,** v. 156, n. 8, p. 1397-413, 2011.

MATTHIJNSSENS, J. et al. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. **Arch Virol,** v. 157, n. 6, p. 1177-82, 2012.

MCNULTY, M. S.; REYNOLDS, D. L. Rotavirus infection. In: SAIF, Y. M. (Ed.). **Disease of Poultry**. 12th. Iowa: Blackwell Publishing, 2008. p.338-355.

MEBUS, C. A. et al. Calf diarrhea (Scours): Reproduced with a virus from a field outbreak. **Research Bulletin: Bulletin of the Agricultural Experiment Station of Nebraska,** v. 233, 1969.

MÉNDEZ, E.; ARIAS, C. F. Astroviruses. In: KNIPE, D. M. e HOWLEY, P. M. (Ed.). **Fields Virology**. 6th. Philadelphia: Lippincott Williams and Wilkins, 2013. p.609-628.

METTIFOGO, E. et al. Emergence of enteric viruses in production chickens is a concern for avian health. **The Scientific World Journal**, p. 1-8, 2014.

MEULEMANS, G.; PEETERS, J. E.; HALEN, P. Experimental infection of broiler chickens with rotavirus. **British Veterinary Journal,** v. 141, p. 69-73, 1985.

MIHALOV-KOVACS, E. et al. Candidate new rotavirus species in sheltered dogs, Hungary. **Emerg Infect Dis,** v. 21, n. 4, p. 660-3, 2015.

MILLER, R. R. et al. Metagenomics for pathogen detection in public health. **Genome Med,** v. 5, n. 9, p. 81, 2013.

MOKILI, J. L. et al. Identification of a novel human papillomavirus by metagenomic analysis of samples from patients with febrile respiratory illness. **PLoS One,** v. 8, n. 3, p. e58404, 2013.

MOKILI, J. L.; ROHWER, F.; DUTILH, B. E. Metagenomics and future perspectives in virus discovery. **Curr Opin Virol,** v. 2, n. 1, p. 63-77, 2012.

MONTGOMERY, R. D. et al. Attempts to reproduce a runting/stunting-type syndrome using infectious agents isolated from affected Mississippi broilers. **Avian Diseases,** v. 4, p. 80-92, 1997.

MOR, S. K. et al. Molecular characterization of L class genome segments of a newly isolated turkey arthritis reovirus. **Infect Genet Evol,** v. 27, p. 193-201, 2014.

MORAES, M. P.; COSTA, P. R. S. Parvoviridae. In: FLORES, E. (Ed.). **Virologia Veterinária.** Santa Maria: Editora UFSM, 2007. p.375-396.

MORENO, P. S. et al. Characterisation of the canine faecal virome in healthy dogs and dogs with acute diarrhoea using shotgun metagenomics. **PLoS One,** v. 12, n. 6, p. e0178433, 2017.

NUÑEZ, L. F. N. et al. Detection of enteric viruses in pancreas and spleen of broilers with runting-stunting syndrome (RSS). **Pesquisa Veterinária Brasileira,** v. 36, n. 7, p. 595-599, 2016.

OLSEN, D. E. Isolation of a reovirus-like agent from broiler chicks with diarrhea and shunting. **Proceedings of the 26th Western Poultry Diseases Conference**, p. 131-139, 1977.

OLUWAYELU, D. O.; TODD, D. Chicken astrovirus infection: minireview and preliminary serologic evidence of antigenically and genetically distinct chicken astroviruses in nigerian indigenous chickens. **Afr. J. Biomed. Res.,** v. 15, p. 71-76, 2012.

OTTO, P. et al. Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). **Avian Dis,** v. 50, n. 3, p. 411-8, 2006.

OTTO, P. H. et al. Detection of avian rotaviruses of groups A, D, F and G in diseased chickens and turkeys from Europe and Bangladesh. **Vet Microbiol,** v. 156, n. 1-2, p. 8-15, 23 2012.

PAGE, R. K. et al. Malabsorption syndrome in broiler chickens. **Avian Diseases,** v. 26, p. 618-624, 1982.

PALADE, E. A. et al. Naturally occurring parvoviral infection in Hungarian broiler flocks. **Avian Pathol,** v. 40, n. 2, p. 191-7, 2011.

PALLEN, M. J. Diagnostic metagenomics: potential applications to bacterial, viral and parasitic infections. **Parasitology,** v. 141, n. 14, p. 1856-62, 2014.

PANTIN-JACKWOOD, M. J. et al. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. **Avian Dis,** v. 52, n. 2, p. 235-44, 2008.

PANTIN-JACKWOOD, M. J.; SPACKMAN, E.; WOOLCOCK, P. R. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. **Avian Dis,** v. 50, n. 3, p. 397-404, 2006.

PANTIN-JACKWOOD, M. J. et al. Molecular characterization of avian astroviruses. **Arch Virol,** v. 156, n. 2, p. 235-44, 2011.

PANTING-JACKWOOD, M. J. et al. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. **Avian Diseases,** v. 51, n. 3, p. 674–680, 2007.

PAULY, M. et al. Molecular epidemiology of Avian Rotaviruses Group A and D shed by different bird species in Nigeria. **Virol J,** v. 14, n. 1, p. 111, 2017.

PHAN, T. G. et al. Rotavirus I in feces of a cat with diarrhea. **Virus Genes,** v. 53, n. 3, p. 487-490, 2017.

POSADA-CESPEDES, S.; SEIFERT, D.; BEERENWINKEL, N. Recent advances in inferring viral diversity from high-throughput sequencing data. **Virus Res,** v. 239, p. 17-32, 2017.

QAMAR, M. F.; ASLAM, H.; JAHAN, N. Histopathological studies on stunting syndrome in broilers, lahore, pakistan. **Vet Med Int,** v. 2013, p. 212830, 2013.

REBEL, J. M. J. et al. Malabsorption syndrome in broilers. **World's Poultry Science Journal,** v. 62, p. 17-30, 2006.

REECE, R. L.; FRAZIER, J. A. Infectious stunting syndrome of chickens in Great Britain: field and experimental studies. **Avian Pathol,** v. 19, n. 4, p. 723-58, 1990.

REKIK, R. M.; SILIM, A.; BERNIER, G. Experimental infection of broiler chickens using different serotypes of reovirus from malabsorption syndrome cases. **Proceedings of the 36th Western Poultry Disease Conference**, p. 146-148, 1987.

RIESENFELD, C. S.; SCHLOSS, P. D.; HANDELSMAN, J. Metagenomics: Genomics analysis of microbial communities. **Annual Review of Genetics,** v. 38, p. 525-552, 2004.

ROSARIO, K.; BREITBART, M. Exploring the viral world through metagenomics. **Curr Opin Virol,** v. 1, n. 4, p. 289-97, 2011.

ROSARIO, K.; DUFFY, S.; BREITBART, M. A field guide to eukaryotic circular single-stranded DNA viruses: insights gained from metagenomics. **Arch Virol,** v. 157, n. 10, p. 1851-71, 2012.

SAIF, Y. M. Viral Enteric Infections. In: SAIF, Y. M. (Ed.). **Disease of Poultry.** 12. Iowa: Blackwell Publishing,, 2008. p.329-355.

SAIKAWA, T. et al. Neutralizing linear epitopes of B19 parvovirus cluster in the VP1 unique and VP1-VP2 junction regions. **J Virol,** v. 67, n. 6, p. 3004-9, 1993.

SANGER, F.; NICKLEN, S.; COULSON, A. R. DNA sequencing with chain-terminating inhibitors. **Proc Natl Acad Sci U S A,** v. 74, n. 12, p. 5463-7, 1977.

SCHMIEDER, R.; EDWARDS, R. Quality control and preprocessing of metagenomic datasets. **Bioinformatics,** v. 27, n. 6, p. 863-4, 2011.

SCHUMANN, T. et al. Evidence of interspecies transmission and reassortment among avian group A rotaviruses. **Virology,** v. 386, n. 2, p. 334-43, 2009.

SELL, J. L.; REYNOLDS, D. L.; JEFFREY, M. Evidence that bacteria Are Not Causative Agents of Stunting Syndrome in Poults. **Poultry Science,** v. 71, p. 1480-1485, 1992.

SELLERS, H. et al. A purified recombinant baculovirus expressed capsid protein of a new astrovirus provides partial protection to runting-stunting syndrome in chickens. **Vaccine,** v. 28, n. 5, p. 1253-63, 2010.

SHAH, J. D. et al. Comparison of tissue sample processing methods for harvesting the viral metagenome and a snapshot of the RNA viral community in a turkey gut. **J Virol Methods,** v. 209, p. 15-24, 2014.

SHAN, T. et al. The fecal virome of pigs on a high-density farm. **J Virol,** v. 85, n. 22, p. 11697-708, 2011.

SHAPIRO, F.; NIR, I. Stunting syndrome in broilers: physical, physiological, and behavioral aspects. **Poult Sci,** v. 74, n. 1, p. 33-44, 1995.

SHAPIRO, F.; NIR, I.; HELLER, D. Stunting syndrome in broilers: effect of stunting syndrome inoculum obtained from stunting syndrome affected broilers, on broilers, leghorns and turkey poults. **Poult Sci,** v. 77, n. 2, p. 230-6, 1998.

SHARPTON, T. J. An introduction to the analysis of shotgun metagenomic data. **Front Plant Sci,** v. 5, p. 209, 2014.

SINGH, J. et al. Metagenomics: Concept, methodology, ecological inference and recent advances. **Biotechnol J,** v. 4, n. 4, p. 480-94, 2009.

SMART, I. J. et al. Experimental reproduction of the runting-stunting syndrome of broiler chickens. **Avian Pathol,** v. 17, n. 3, p. 617-27, 1988.

SMYTH, V. J. A Review of the Strain Diversity and Pathogenesis of Chicken Astrovirus. **Viruses,** v. 9, n. 2, 2017.

SONGSERM, T. et al. A comparative study of the pathogenesis of malabsorption syndrome in broilers. **Avian Dis,** v. 44, n. 3, p. 556-67, 2000.

SONGSERM, T. et al. Experimental reproduction of malabsorption syndrome with different combinations of reovirus, Escherichia coli, and treated homogenates obtained from broilers. **Avian Dis,** v. 46, n. 1, p. 87-94, 2002.

SVRAKA, S. et al. Metagenomic sequencing for virus identification in a public-health setting. **J Gen Virol,** v. 91, n. Pt 11, p. 2846-56, 2010.

TARASIUK, K.; WOZNIAKOWSKI, G.; SAMOREK-SALAMONOWICZ, E. Occurrence of chicken parvovirus infection in poland. **Open Virol J,** v. 6, p. 7-11, 2012.

THOMAS, T.; GILBERT, J.; MEYER, F. Metagenomics - a guide from sampling to data analysis. **Microb Inform Exp,** v. 2, n. 1, p. 3, 2012.

THURBER, R. V. et al. Laboratory procedures to generate viral metagenomes. **Nat Protoc,** v. 4, n. 4, p. 470-83, 2009.

TIJSSEN, P. et al. Parvoviridae. In: KING, A. M. Q.;ADAMS, M. J., *et al* (Ed.). **Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses**: Academic Press Elsiever, 2012. p.405-425.

TINÔCO, I. F. F. Avicultura industrial: novos conceitos de materiais, concepções e técnicas construtivas disponíveis para galpões avícolas brasileiros. **Revista Brasileira de Ciência Avícola,** v. 3, 2001.

TROJNAR, E. et al. The genome segments of a group D rotavirus possess group A-like conserved termini but encode group-specific proteins. **J Virol,** v. 84, n. 19, p. 10254-65, 2010.

VAN DER HEIDE, L. The history of avian reovirus. **Avian Diseases,** v. 44, p. 638-341, 2000.

VAN LOON, A. A. et al. Isolation of a new serotype of avian reovirus associated with malabsorption syndrome in chickens. **Veterinary Quarterly,** v. 23, p. 129-133, 2001.

VICTORIA, J. G. et al. Metagenomic analyses of viruses in stool samples from children with acute flaccid paralysis. **J Virol,** v. 83, n. 9, p. 4642-51, 2009.

WANG, H. et al. Plasma virome of cattle from forest region revealed diverse small circular ssDNA viral genomes. **Virol J,** v. 15, n. 1, p. 11, 2018.

WILLNER, D. et al. Metagenomic analysis of respiratory tract DNA viral communities in cystic fibrosis and non-cystic fibrosis individuals. **PLoS One,** v. 4, n. 10, p. e7370, 2009.

WOOLEY, J. C.; GODZIK, A.; FRIEDBERG, I. A primer on metagenomics. **Plos Computacional Biology,** v. 6, p. 1-13, 2010.

YANG, J. et al. Unbiased parallel detection of viral pathogens in clinical samples by use of a metagenomic approach. **J Clin Microbiol,** v. 49, n. 10, p. 3463-9, 2011.

YASON, C. V.; SCHAT, K. A. Experimental infection of specific pathogen free chickens with avian rotaviruses. **Avian Diseases,** v. 30, p. 551-556, 1986.

ZAVALA, G.; BARBOSA, T. Runting and shunting in broiler chickens. **Apinco-Facta**, p. 1-4, 2006.

ZAVALA, G.; SELLERS, H. Runting/stunting syndrome. **The Informed Poultry Professional Issue,** v. 85, p. 1-4, 2005.

ZHANG, B. et al. Viral metagenomics analysis demonstrates the diversity of viral flora in piglet diarrhoeic faeces in China. **J Gen Virol,** v. 95, p. 1603-11, 2014.

ZHANG, W. et al. What is for dinner? Viral metagenomics of US store bought beef, pork, and chicken. **Virology,** v. 468-470, p. 303-10, 2014.

ZHANG, Y. et al. Complete genome sequence of a novel avastrovirus in goose. **Arch Virol,** v. 162, n. 7, p. 2135-2139, 2017.

ZSAK, L.; CHA, R. M.; DAY, J. M. Chicken parvovirus-induced runting-stunting syndrome in young broilers. **Avian Dis,** v. 57, n. 1, p. 123-7, 2013.

ZSAK, L.; STROTHER, K. O.; DAY, J. M. Development of a polymerase chain reaction procedure for detection of chicken and turkey parvoviruses. **Avian Dis,** v. 53, n. 1, p. 83-8, 2009.

ANEXO A – Parecer favorável à pesquisa concedido pelo CEUA – IPVDF.

Fepagro

Secretaria da Agricultura, Pecuária e Agronegócio

Rio Grande do Sul
Governo do Estado

Ofício n.º 24/14 – CEUA/IPVDF Eldorado do Sul, 11 de dezembro de 2014.

Senhora Pesquisadora:

Com relação ao Projeto de Pesquisa – CARACTERIZAÇÃO DO VIROMA ASSOCIADO AO TRATO ENTÉRICO DE AVES PROVENIENTES DE DIFERENTES REGIÕES DO ESTADO DO RIO GRANDE DO SUL ATRAVÉS DA ANÁLISE METAGENÔMICA – protocolado para análise e parecer sob nº. 21/2014, por Diane Alves de Lima, em 19 de novembro de 2014, cabe referir o seguinte:

A Comissão de Ética no Uso de Animais (CEUA-IPVDF) reuniu-se extraordinariamente em 26 de novembro e 11 de dezembro de 2014, no Instituto de Pesquisas Veterinárias Desidério Finamor e, após as solicitações feitas terem sido atendidas, emite **PARECER FAVORÁVEL** à respectiva pesquisa a ser desenvolvida, em 600 frangos de corte, cuja parte experimental abrange o período de janeiro de 2015 ao primeiro semestre de 2017.

Atenciosamente,

Fernando Sérgio Castilhos Karam
Coordenador CEUA - IPVDF