News on *Actinobacillus pleuropneumoniae* -
our diagnostics are at the cutting edge of science!

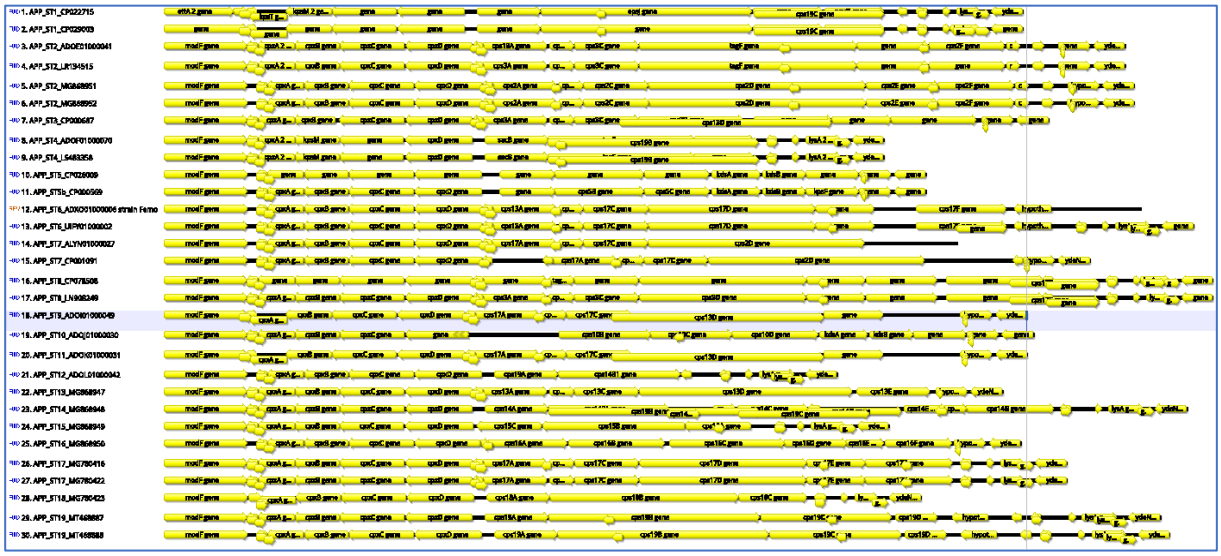

Figure 1:
Cps-Loci of App 1-19 (in numeric order, not sorted by homologies), © IVD 2021

Scientific experts identified a new ***Actinobacillus pleuropneumoniae (App)* serotype 19** and updated the established multiplex PCR (mPCR) for capsule-specific typing ("molecular serotyping") of all known *App* serotypes (Stringer et al. 2021a). Primers for species identification based on the a*pxIV* gene were also optimised.

Based on these results it becomes clear that in the recent past some misclassifications regarding the identified *App*-(capsule)-serotype occurred by means of the *cps*-mPCR by Bosse et al. (2018), e.g. with *App* serotype 8 and the so far not identifiable *App* serotype 19.

Furthermore, the serotype-defining *cps* locus of *App* serotypes 9 and 11 is shown to differ only in a single base in the *cpsF* gene, so that they cannot be differentiated. Since *App* 9 and 11 also have the same LPS-O antigen locus as well as the same Apx profile, it is proposed that the two serotypes be regarded as hybrid serotypes 9/11.

Retrospectively, we were able to identify a previously untypeable isolate from 2018 as *App* 19 in our own initial investigations using optimised cps-mPCR (Stringer et al. 2021).

In general, isolates of the same serotype are coding for the same LPS-O-antigens, with some serotypes having the same or very similar O-antigens leading to well-known serologic cross-reactions, e.g. serogroups 3/6/8/15, 4/7 and 1/9/11 (Gottschalk, 2015).

Atypical *App* variants are characterised by the fact that the isolates differ in biotype (NAD-dependent growth), LPS-O antigen type and/or Apx toxin profile from the respective reference strains of the capsule types (former: serotypes). Recent studies (Schuwerk et al. 2021) show that the proportion of atypical variants may not be insignificant (>70%, but n=4 or 7).

Previous attempts to isolate *App* in samples and simultaneously characterise the pathogen in terms of serotype and virulence type have largely failed. However, the same renowned research group that newly identified *App* (serotype) 19 (see above) was recently able to optimise a method in which lung samples from pigs could be typed directly with the new cps-mPCR by Stringer et al. (2021).

For this purpose, suitable lung samples or *App*-isolates are applied to FTA® cards (Whatman™). This offers advantages for shipping these samples, as the pathogen is inactivated and therefore no longer infectious

when applied to the sample cards. Further, these sample cards do not have to be refrigerated. However, by using FTA® cards, only DNA is preserved and in limited quantities, whereas archived isolates can usually be accessed again and again through renewed cultivation.

Conclusions for diagnostics:

1. Diagnostics must continue to be adapted to latest scientific findings in the future. It can be expected that in course of increasing genome analyses further types and variants of *App* will be identified.
2. According to the current status, all ***App*-isolates** should be analysed by the new **cps-mPCR** by Stringer et al. (2021) to ensure the best possible identification of the capsule type (former: serotype) which is of great importance for the selection of appropriate vaccines as well as for the epidemiologic evaluation.
3. Due to the increasing occurrence of atypic *App*-variants the **virulence potential** can be inferred even less than before from the capsule type. It is therefore advisable to additionally establish the **Apx-toxin-profile** by means of mPCR for all isolates to assess the virulence potential.
4. The biotype is determined by **classical bacterial cultivation** as well as by **cps-mPCR** using the nadV gene. Confirmation of the ***App* species** is ensured by the apxIV target in the cps-mPCR.
5. Capsule-(sero)-typing by cps-mPCR according to Stringer et al. (2021a) directly on suitable lung samples applied on FTA®-cards has now been validated for the first time (Stringer et al. 2021b).

⇨We will establish and evaluate the procedure and also test it with regard to Apx-toxin typing!

Our diagnostics are state of the art!
The following diagnostic options are available at the IVD GmbH:

- **Direct PCR detection** of *App* in suitable sample material using ***App* PCR** (*App* ApxIV PCR) based on the ApxIV toxin gene.
- **Culture cultivation** and isolation of *App* isolates from fresh sample material, e.g. after necropsy.
- **Molecular serotyping** of *App* isolates by ***App* capsule typing multiplex PCR (*App* cps-mPCR)** updated after Stringer et al. (2021).
- Assessment of the **virulence potential** of *App* isolates using ***App* toxin typing multiplex PCR** for determination of the Apx toxin profile.

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