News about *Actinobacillus pleuropneumoniae* - our diagnostics are up to date with the latest scientific findings!

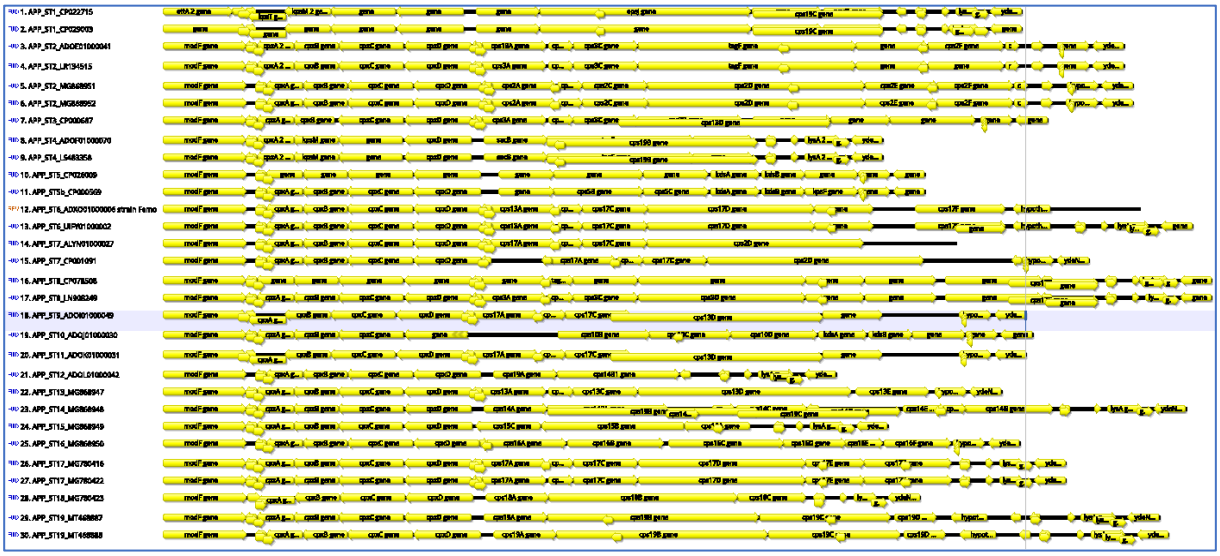


Figure 1: Cps-Loci from App serovars 1-19 (in numerical order, not sorted by homologies), © IVD 2021

Experts on ***Actinobacillus pleuropneumoniae (App)*** have identified another new **serovar 19 (*App* 19)** and have adapted and updated the previously established multiplex PCR (mPCR) for capsule-specific typing ("molecular serotyping") of all known serovars (synonymous with serotypes) to the new findings (Stringer et al. 2021a).

Based on these results, it is clear that there have also been some recent misclassifications regarding the identified (capsule) serovars using the previously used cps-mPCR according to (Bosse et al. 2018) for App, e.g., for App serovars 8 and the previously unidentifiable *App 19*.

Primers for species detection based on the *apx*IV gene were also optimized.

Furthermore, the serovar-defining *cps* locus of *App* serovars 9 and 11 is shown to differ only in a single base in the *cpsF* gene, so that they cannot be differentiated. Furthermore, since *App* 9 and 11 also share the same LPS-O antigenic locus as well as the same Apx toxin profile, it is suggested that the two serovars are hybrid serovars 9/11.

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

In general, isolates of the same serovar also encode the same LPS O antigens, however, some different serovars also have the same or very similar O antigens, which can lead to the well-known serological cross-reactions, e.g., in serogroups 3/6/8/15, 4/7, and 1/9/11 (Gottschalk, 2015).

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

Previous attempts to detect *App* in samples and at the same time to characterize the pathogen in terms of serovar and virulence type have largely failed. However, the same renowned research group that newly identified *App* (serovar) 19 (see above) has now recently been able to optimize a method in which immediate typing of App contained in the sample is also possible using lung samples from pigs by means of the new cps-mPCR (Stringer et al. 2021a and b).

Suitable lung samples or *App* isolates are applied to FTA® cards (Whatman™) for this purpose, which also offers advantages for shipping these samples, as the pathogen is inactivated when applied to the sample cards and is therefore no longer infectious, and these sample cards do not need to be refrigerated.
On the other hand, when using FTA® cards, only DNA is conserved and in limited quantities, whereas archived *App* isolates can usually be accessed again and again through renewed cultivation.

Conclusion for diagnostics from the latest findings:

1. Diagnostics must continue to be adapted to scientific findings in the future. It is to be expected that more types and variants of *App* will be identified in the course of increased genome analyses.
2. As things stand today, **all *App* isolates** should be analysed with the new **cps-mPCR** according to Stringer et al. 2021. This ensures the best possible identification of the capsule type (old: serotype/serovar) and this is of great importance both for the selection of suitable vaccines and for an epidemiological evaluation.
3. Due to the increased occurrence of atypical variants, the **virulence potential** can be inferred even less than before from the capsule type (old: serotype/saerovar). It is therefore advisable to additionally establish the **Apx toxin profile** by means of **apx-mPCR** for all isolates in order to be able to derive an estimate of 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. Die Bestimmung des Biotyps erfolgt zum einen durch die **klassische, bakterielle Kultivierung** als auch im Rahmen der **cps-mPCR** anhand des nadV-Gens. Die Bestätigung **der Spezies *App*** wird durch das apxIV-Target in der cps-mPCR gewährleistet.
6. Capsule (sero-)typing by cps-mPCR according to Stringer et al. (2021a) directly on suitable lung samples applied to FTA® cards has now been validated for the first time (Stringer et al. 2021b).
   ⇨ We will establish and evaluate the method in our company and also test it with regard to Apx toxin typing!

Our diagnostics are state of the art!
The following diagnostic options are available at IVD GmbH:
Bei der IVD GmbH stehen folgende diagnostische Möglichkeiten zur Verfügung:

- **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 dissection.
- **Molecular serotyping** of *App* isolates by ***App* capsule typing multiplex PCR** **(*App* cps-mPCR)** updated according to Stringer et al. 2021.
- Assessment of the **virulence potential** of *App* isolates using the ***App* Toxin Typing Multiplex PCR** **(*App* apx-mPCR)** for Apx toxin profiling.

Please do not hesitate to contact me for further information.

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